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Volume I

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THE EFFECTS OF OZONATION REACTIONS IN WATER

Volume I

Final Report

March 1979

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16. ABSTRACT

The potential biological hazard of ozonation was evaluated by using rapid microbial bioassays of selected organic compounds under ozonation conditions. Twenty-nine compounds were evaluated for mutagenic activity before and after ozonation using the Ames Salmonella/microsome and the yeast Saccharomyces cerevisiae D3 assays.

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SUMMARY

Of the 29 compounds investigated in this study, 7 were mutagenic after ozonation. Ethanol, nitrilotriacetic acid, glycerol, benzidine, hydroquinone, and phenol showed moderate to weak mutagenic activity in the *Saccharomyces cerevisiae* D3 yeast system, and 1,1-diphenylhydrazine, ethanol, glycerol, and benzidine showed moderate to weak activity in two *Salmonella typhimurium* strains.

The mutagenic activity resulting from ozonation of ethanol can be attributed to the formation of hydroperoxides, which as a class tend to show both toxic and mutagenic properties in *S. cerevisiae* D3. Hydroperoxides also may form during the ozonation of glycerol.

Benzidine, which is a known mutagen when metabolically activated (post-mitochondrial liver homogenate) in *Salmonella*, showed mutagenic activity without activation after ozonation. This result is important because in the yeast system, *S. cerevisiae*, benzidine showed no activity before ozonation, even in the presence of metabolic activation, but did show activity in post-ozonated solutions.

The pH of each solution was found to decrease on ozonation, and this had an effect on the distribution of products of 1,1-diphenylhydrazine. This decrease in pH may also affect the lifetimes of potential mutagens, such as epoxides and lactones, which may decompose at low pH values.

The combined chemical and mutagenic screening data appear in Table 1.

Table 1

SUMMARY OF RESULTS FROM MUTAGENICITY TESTING*

Compound	Salmonella typhimurium		Saccharomyces cerevisiae D3	
	Pre-ozonation	Post-ozonation	Pre-ozonation	Post-ozonation
2,4-Dinitrotoluene	-	-	-	-
Benzoic acid	-	-	-	-
Ethanol	-	+	-	+
Benzo(a)pyrene	-	-	-	-
HMX	-	-	-	-
RDX	-	-	-	-
Oleic acid	-	-	-	-
Humic acid	-	-	-	-
TNT condensate water	+	-	-	-
1,1-Diphenylhydrazine	-	-	-	-
Nitrilotriacetic acid, trisodium salt monohydrate	-	-	-	+
Aldrin	-	-	-	-
DDT	-	-	-	-
Aroclor 1254	-	-	-	-
Acetic acid	-	-	-	-
Glucose	-	-	-	-
Urea	-	-	-	-
Cholesterol	-	-	-	-

---

* In the columns, (-) indicates a negative mutagenic response, and (+) indicates a mutagenic response.

Table 1 (Concluded)

Compound	Salmonella typhimurium		Saccharomyces cerevisiae D3	
	Pre-ozonation	Post-ozonation	Pre-ozonation	Post-ozonation
Benzidine•2 HCl	+	+	−	+
Glycine	−	−	−	−
Benzene	−	−	−	−
Cysteine	−	−	−	−
Thymine	−	−	−	−
Caffeine	−	−	−	−
Diethylamine	−	−	−	−
Phenol	−	−	−	+
Hydroquinone	−	−	−	+
Alanylglycylglycine	−	−	−	−
Glycerol	−	+	−	+

INTRODUCTION

The use of chlorine in the water purification process recently has come under critical review because low levels of chlorinated organics have been found in drinking water. The potential biological hazard that these chemicals present to man is unknown, but some of the materials are established as carcinogens in animal studies.

These findings have led to the search for alternatives to chlorination for water purification. Ozonation is one alternative, a process that has been used in Europe for many years. However, the biological effects of the products of ozonation are unkown and should be evaluated before ozonation is considered as a replacement for chlorination in the United States.

In this study, we evaluated the potential biological hazard of ozonation by using rapid microbial bioassays of selected organic compounds under ozonation conditions. The results represent a preliminary estimate of the formation of biologically active compounds produced by this water treatment technique.

Chemicals were chosen to represent various classes of chemicals known to occur in drinking water, as well as chemicals expected to be present in water reuse systems, e. g., self-contained, transportable medical units (MUST). The chemicals tested were 2,4-dinitrotoluene, benzoic acid, ethanol, benzo(a)pyrene, HMX, RDX, oleic acid, humic acids, TNT condensate water, 1,1-diphenylhydrazine, nitrilotriacetic acid trisodium salt monohydrate, aldrin, DDT, aroclor 1254, acetic acid, glucose, urea, cholesterol, benzidine, glycine, benzene, cysteine, thymine, caffeine, diethylamine, phenol, hydroquinone, alanylglycylglycine, and glycerol.

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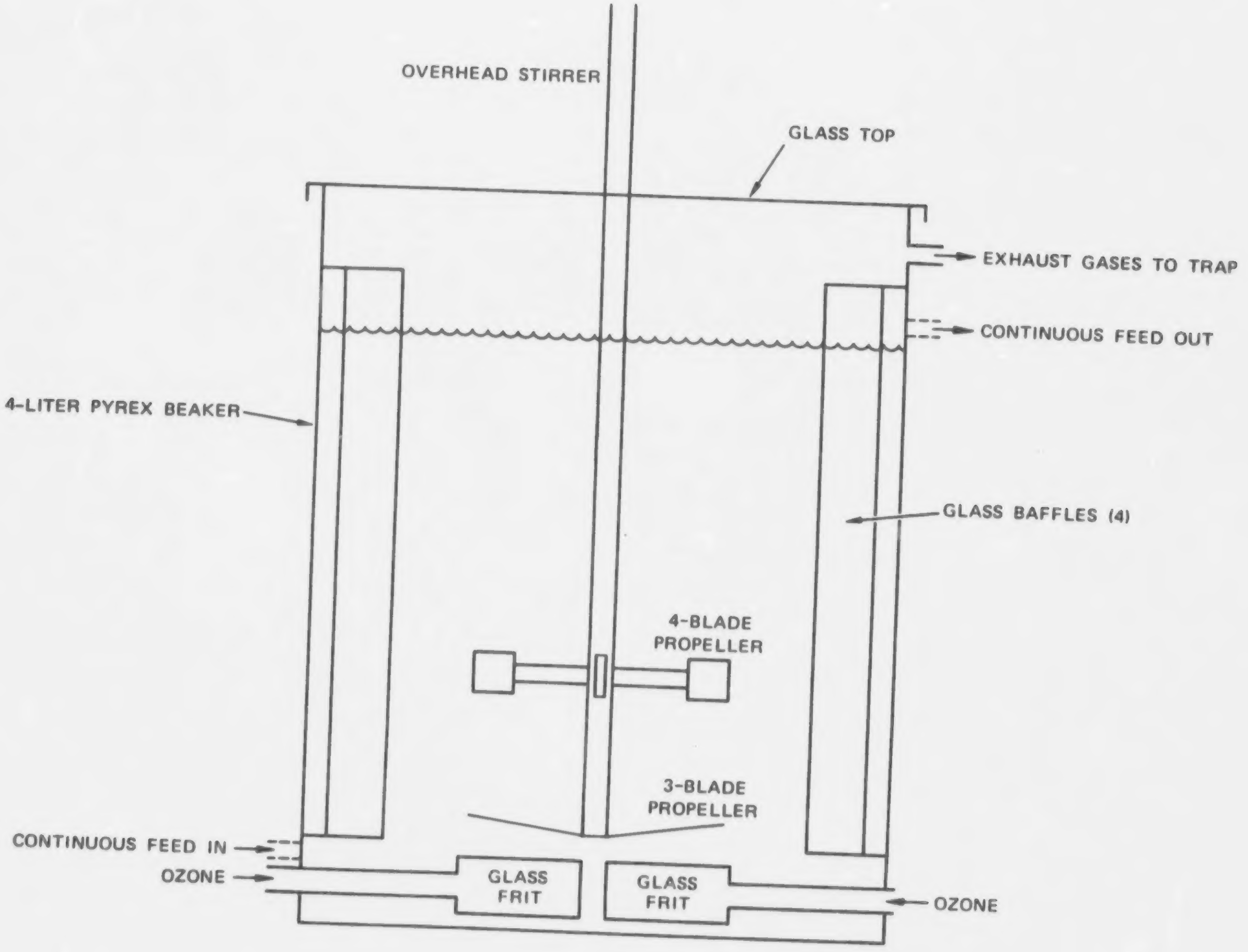
The ozonations were performed on aqueous solutions of the above compounds using a commercial ozone generator (Welsbach). Each solution was analyzed for the parent compound under pre- and post-ozonation conditions and submitted for biological assay.

The biological assays used to determine if ozonation produced biologically active (i.e., mutagenic) products were the Ames *Salmonella*/microsome assay--reversion to histidine independence in five strains of *Salmonella typhimurium*--and mitotic recombination in the yeast *Saccharomyces cerevisiae* D3. A rat liver post-mitochondrial supernatant fraction was incorporated into the assay procedure to provide mammalian metabolic pathways.

Volume 2 of this report describes similar studies utilizing chlorine dioxide as the chemical oxidant.


OVERHEAD STIRRER
GLASS TOP
EXHAUST GASES TO TRAP
CONTINUOUS FEED OUT
4-LITER PYREX BEAKER
GLASS BAFFLES (4)
4-BLADE PROPELLER
3-BLADE PROPELLER
CONTINUOUS FEED IN
OZONE
GLASS FRIT
GLASS FRIT
OZONE

TA-325583-31

FIGURE 1 SCHEMATIC DIAGRAM OF OZONATION REACTION VESSEL

METHODS

Chemical Methods

The objectives of the chemistry section of this study were to provide aqueous solutions of selected organic compounds for mutagenic screening under pre- and post-ozonation conditions, achieve 50% degradation levels of the primary component subjected to ozonation, and identify organics formed in biologically active solutions. To achieve sufficient concentrations of intermediate products for biological assays, starting concentrations of 1% or maximum aqueous solubility were used.

Ozonations were performed in the all-Pyrex® glass reactor shown in Figure 1. The reactor was equipped with a high-speed overhead stirrer and four Pyrex baffles (to prevent vortexing). Ozone, generated from a Welsback Model T-408 ozonator, was fed into the reactor from two all-glass frits under the following instrument settings:

- Pressure, 7.4 lb/in^2
- Flow, 1.0 standard liters/min
- Voltage, 100 volts
- Ozone rate, 0.141 mol/hr (6.78 g/hr)

The ozone rates were varied slightly to accommodate slow- or fast-reacting materials. Ozone concentrations were determined by standard iodometric titrations.

The reactions were monitored by use of a Varian 2700 gas chromatograph (gc) equipped with flame-ionization detectors and coupled to a Hewlett Packard 3380A integrator-recorder or by use of high-pressure liquid chromatography (hplc). For hplc, we used a Spectra-Physics Model 35008 liquid chromatograph (lc) equipped with uv or fluorescence detectors and coupled to a Spectra-Physics Minigrator and Omniscribe recorder.

Most gc profiles were obtained using a 1.8 m x 2 mm glass column

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packed with 10% DC-200 on Gas Chrom Q. Liquid chromatographic profiles were performed on a Water's C_{18}-μ Bondapack column (reverse phase) using a 75% methanol-water solvent system. Quantitations were performed by the internal standard method. Specific chromatographic conditions appear in Appendix A.

The pH of each solution was measured with an Orion Research 701 digital pH meter.

Products were identified by use of an LKB 9000 gas chromatograph/mass spectrometer (gc/ms) equipped with a PDP-12 computer. Identifications were confirmed by comparing the spectra of the products with those of reference compounds or authentic spectra. The compounds studied were obtained from commercial sources, in the highest available purity. Chromatographic analysis showed all compounds to be > 98% pure.

The combined chemical and mutagenic screening data are presented in the Appendix. Selected conditions for the ozonation of the individual organics are described under "Chemical Studies" in the Results Section to clarify the chemical discussions.

Microbiological Methods

Mutagenic Assays with Salmonella typhimurium

The *Salmonella typhimurium* strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The *his*$^+$ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley.[1-3] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa$^-$) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of vitamin biotin (bio$^-$) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB$^-$). The rfa$^-$ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of those molecules. The uvrB$^-$ mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[5] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. We have shown that TA100 can detect mutagens such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2) in plate assays more easily than can TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens (e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene). Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthracenes, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All the indicator strains are rountinely checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. Cultures are then stored in 10% sterile glycerol at -80° C. For each experiment, an inoculum from the stock cultures is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals, either of the aromatic amino type or polycyclic hydrocarbon type, are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[4,6,7] Some of these intermediate metabolites are very potent mutagens in the *S. typhimurium* test. Ames has described the liver metabolic activation system that we use.[6] In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection the animals' food is removed but drinking water is provided *ad libitum*. On the fifth day, the rats are sacrificed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x *g*, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*

(2) 0.05 ml of indicator organisms

(3) 0.50 ml of metabolic activation mixture (optional)

(4) Up to 0.25 ml of a solution of the test chemical.

For negative controls, we use steps (1), (2), and (3) (optional). For positive controls, we test each culture by specific mutagens known to revert each strain using steps (1), (2), (3) (optional), and (4).

This mixture is stirred gently and then poured onto minimal agar plates.† After the top agar has set, the plates are incubated at 37° C for 2 days. The number of *his*$^+$ revertant colonies is counted and recorded.

Mutagenic Assays with Saccharomyces cerevisiae

The yeast *S. cerevisiae* D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.[9] When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on the plates.[10]

---

* 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.

† Minimal agar plates consist of, per liter, 15 g of agar, 50 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_4PO_4 \cdot H_2O$.

The S. cerevisiae tester strain is stored at -80° C. For each experiment, the tester strain is inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30° C with aeration.

The *in vitro* yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged, and the cells are resuspended at a concentration of 10^8 cells/ml in a 67 mM phosphate buffer (pH7.4). To a sterile test tube are added:

- 1.00 ml of the organisms
- 0.50 ml of either the metabolic activation mixture or buffer
- 1.00 ml of the test chemical.

The chemicals to be tested are diluted in 0.067 M phosphate buffer. Several doses of the chemical are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30° C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline, and a volume of 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on tryptone-yeast agar plates: five plates are used for the 10^{-3} dilution and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissection microscope at 10 X magnification, and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the 10^{-5} dilution.

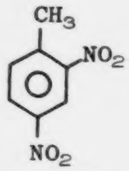
The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

RESULTS

Chemical Studies

The reaction conditions for the preparation of each solution for mutagenic screening are listed in the following sections for each chemical investigated. Also included in this section are some suggested identifications of reaction products.

2,4-Dinitrotoluene

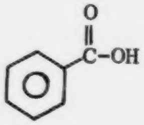


Source: Matheson, Coleman & Bell

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
28.3	9	20	96	8.4	3.8
79.6	6	17	55	8.1	3.5

Quantitations of 2,4-dinitrotoluene were performed by gc. No new products were observed in the profile.

Benzoic Acid



Source: Mallinckrodt Chemical Works

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
3,662	6.8	60	14	4.3	3.9

Benzoic acid was found to be unreactive with ozone. However, some initial color changes (from colorless to orange) were noted

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during the first 5 min of ozonation. No new products were observed in the gc profile.

Ethanol

CH_3CH_2OH

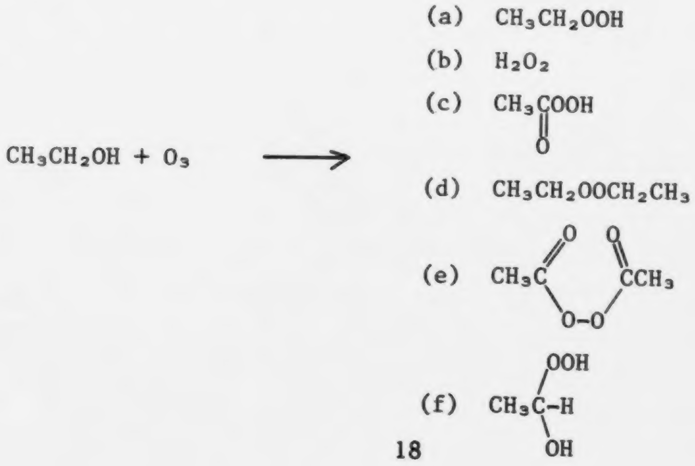
Source: Commercial Solvent Corp.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min)	Amount Reaction (%)	pH Initial	pH Final
7759	9.3	6	73.9	5.35	3.20

Ethanol was found to react with ozone only under stressed conditons. However, since mutagenic activity was found in this sample, detailed chemical investigations were performed.

To determine whether decreases in the ethanol concentration resulted from degassing by ozone, oxygen was allowed to bubble through the reactor for several hours. Quantitative gc analysis of samples before and after degassing showed negligible losses of ethanol.

Figure 2 is the gc profile of ethanol. The products identified by gc/ms were acetaldehyde and acetic acid. An oxidizing agent was formed, which converted iodide to iodine and represented 25.3 mole % of the reacted ethanol. The following peracids, peroxides, and hydroperoxides were considered as possible reaction products [Eq. (1)]:



(1)

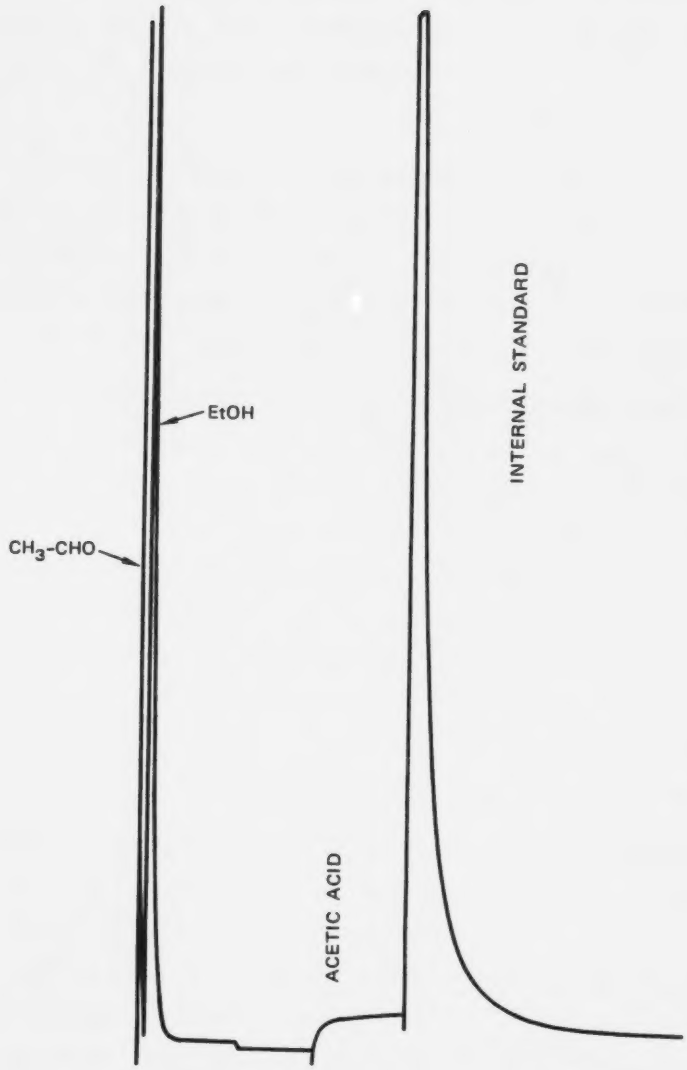


FIGURE 2 GAS CHROMATOGRAPHIC PROFILE OF OZONATED ETHANOL

Treatment of the ozonized solution with lead (IV) tetraacetate produced oxygen gas, a reaction characteristic of alkyl hydroperoxides and hydrogen peroxide but not of dialkyl peroxides, peracids, or diacylperoxides.[11,12] After the solution was treated with lead (IV) tetraacetate, iodide was no longer converted to iodine, indicating the absence of peracids, dialkylperoxides, and diacylperoxides (Compounds c, d, and e).

Ethyl hydroperoxide (Compound a) was synthesized from diethylsulfate and hydrogen peroxide. This compound readily gas chromatographed. However, none of the gc profiles of ozonized ethanol solutions showed the presence of this compound. Therefore, Compound (a) was not considered to be a reaction product.

Hydrogen peroxide (Compound b) was investigated as a reaction product. Since glc and hplc profiles showed no other components besides aldehyde and acetic acid, tlc was used, and the plates were sprayed with potassium iodide for detection of the hydroperoxide. A spot was detected that had an R_f identical to that of hydrogen peroxide. Also, a low-temperature titration of the hydroperoxide with ceric ion, a method reported to distinguish alkyl hydroperoxides from hydrogen peroxide, was positive for H_2O_2. However, biological tests on hydrogen peroxide (the Ames test) indicated much less mutagenic activity than that observed in ozonized ethanol.

We performed the ozonation of ethanol in D_2O to evaluate the nuclear magnetic resonance (nmr) spectrum. During the reaction, a doublet appeared at 1.38 δ, a singlet a 5.0 δ, and a quartet 5.4 δ; also, an acetic acid singlet appeared at 2.1 δ, and a singlet due to water appeared at 4.8 δ. By concentration under vacuum, the ethanol was removed, and integration of the proton signals of the unknowns indicated a ratio of 3:1:1. The ^{13}C-nmr spectrum of the mixture showed three signals for the unknown and two signals for acetic acid. Off-spin resonance of the ^{13}C-nmr signals indicated that the signals arose from methyl, methylene, and methine carbons. Figure 3 is the ^{13}C-nmr spectrum and based on these data, as well as on

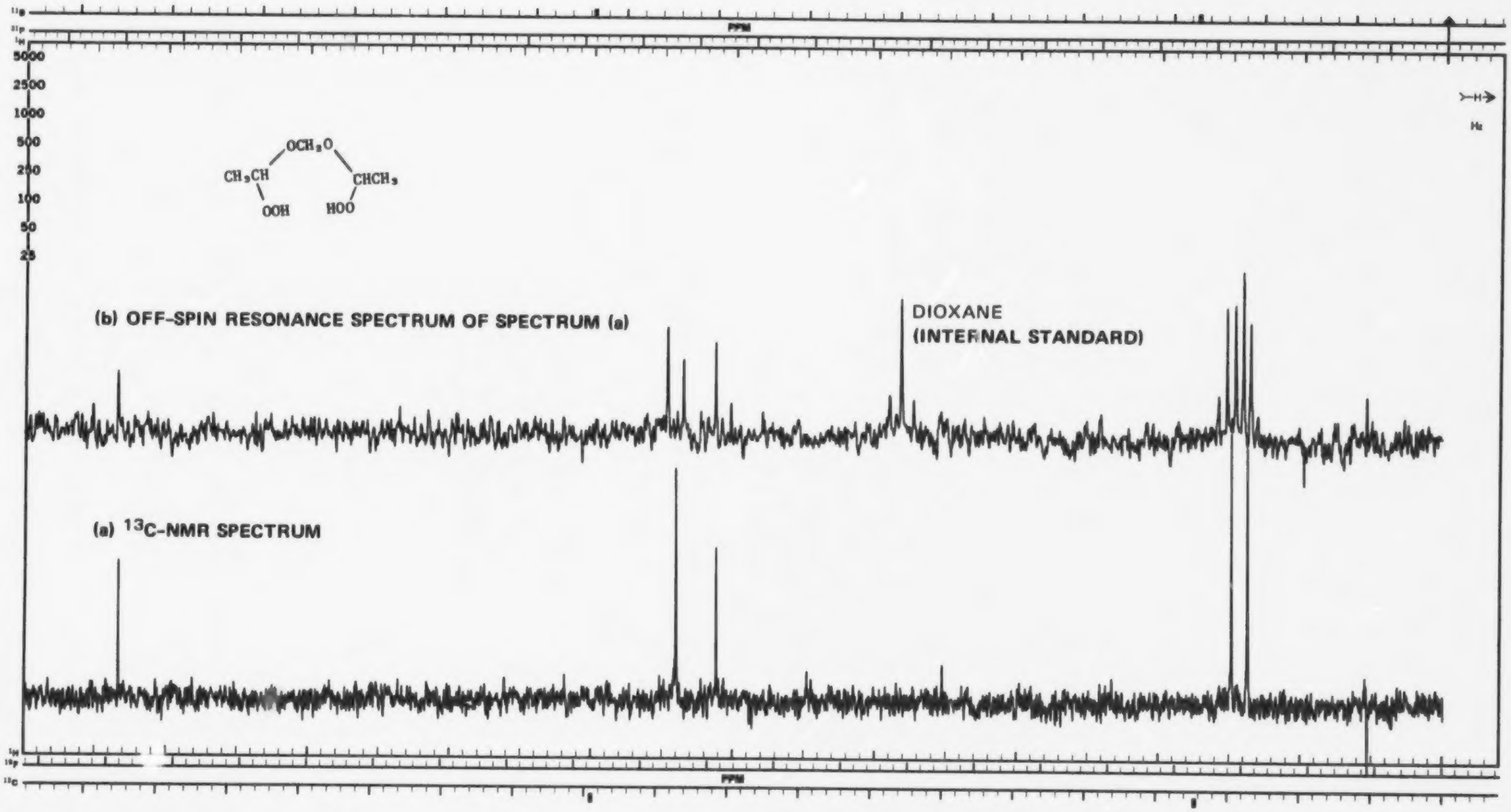
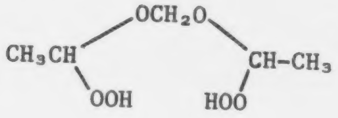


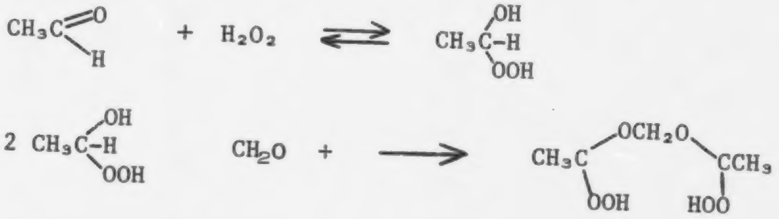
FIGURE 3 ^{13}C-NMR SPECTRA OF REACTION PRODUCTS FROM THE OZONATION OF ETHANOL chemical shifts, the structure of the unknown can be represented as compound I.



I

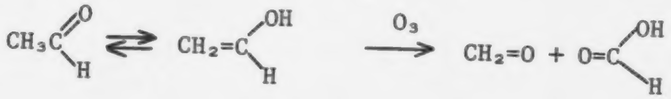
When acetaldehyde, formaldehyde, and hydrogen peroxide were mixed together, proton nmr signals identifical to those of the ozonized ethanol solution were observed.

Compound I appears to be formed from the condensation of a product of the reaction of acetaldehyde and hydrogen peroxide with formaldehyde [Eq. (2)].



(2)

Formaldehyde could arise from the ozonation of the enol form of acetaldehyde Eq. (2) or by a complex free-radical processes on ethylhydroxyhydropenoxide [Eq. (3)] :



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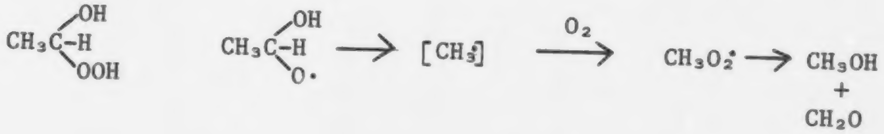
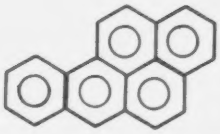


(4)

Eq. (4) may be a more likely mechanism, since no deuterium incorporation in acetaldehyde was detected when the hydroperoxide (I) was destroyed with iodide.

Benzo(α)pyrene (Gold Label)



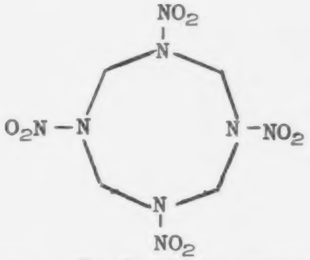
Source: Aldrich Chemical Co.

Initial Conc. (ppb)	Ozone Conc. (g/hr)	Reaction Time (Min)	Amount Reacted (%)	pH Initial	pH Final
0.53	5.5	1	100	6.8	5.9

Benzo(α)pyrene appeared to be very reactive with ozone. However, because of its low solubility in water, little information on product distribution could be established.

Benzo(α)pyrene was monitored by hplc with a fluorescence detector ($\lambda_{max\ excit}=360\lambda_{max\ emm}=408$) having part-per-billion sensitivity.

HMX

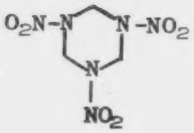


Source: Explosives group, SRI

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min)	Amount Reacted (%)	pH Initial	pH Final
0.3	5.5	75	46	9.15	5.45

HMX underwent ozonation slowly. Solutions were chromatographed by hplc using uv detection at 254 nm. Although minor new products were observed in the hplc profile, none was identified.

RDX



Source: Explosives group, SRI

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min)	Amount Reacted (%)	pH Initial	pH Final
30.8	6.7	30	--	6.35	5.20

RDX failed to react with ozone. Initial and final concentrations were monitored by hplc.

Oleic Acid

$C_8H_{17}CH{=}CH(CH_2)_7COOH$

Source: ICN Pharmaceuticals, Inc.

Oleic acid readily reacted with ozone to yield classical ozonation products. Three major products, identified by gc/ms as their trimethylsilyl derivatives, were $C_8H_{17}COOH$, $OHC{-}(CH_2)_7{-}COOH$, and $HO_2C{-}(CH_2)_7{-}COOH$.

$$C_8H_{17}CH{=}CH(CH_2)_7COOH + O_3 \longrightarrow C_8H_{17}COOH + OHC{-}(CH_2)_7{-}COOH + HO_2C(CH_2)_7CO_2H \qquad (5)$$

Humic Acid

Source: ICN Pharmaceuticals, Inc.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min)	Amount Reacted (%)	pH Initial	pH Final
1-g suspension in 2 liters of H_2O	5.1	20	Solubilization	7.1	3.2

The humic acid materials investigated were highly insoluble in water and showed no initial gc or lc profile. Upon ozonation, all materials became solubilized, but gc and lc profiles were still void of any components. No new products were determined.

TNT Condensate Water

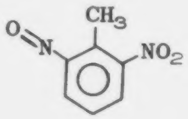
Source: Joliet Army Ammunition Plant

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
35.4	5.1	100	100	7.2	3.6

Condensate water is complex mixture of nitroaromatics produced at TNT manufacturing facilities. The major components are 2,4- and 2,6-dinitrotoluenes. Condensate water is yellow, and this color is rapidly discharged with ozonation.

The extent of reaction was followed using 2,4-dinitrotoluene concentration values for pre- and post-ozonation determinations. Two new products were found but were not identified.

The two new products have mass spectra, shown in Figures 4 and 5, that indicate molecular ions at m/e = 166 and 270. The fractionation pattern of Product 1 (m/e = 166) is consistent with that of 6-nitroso-2-nitrotoluene (II); however, this structure was not confirmed.



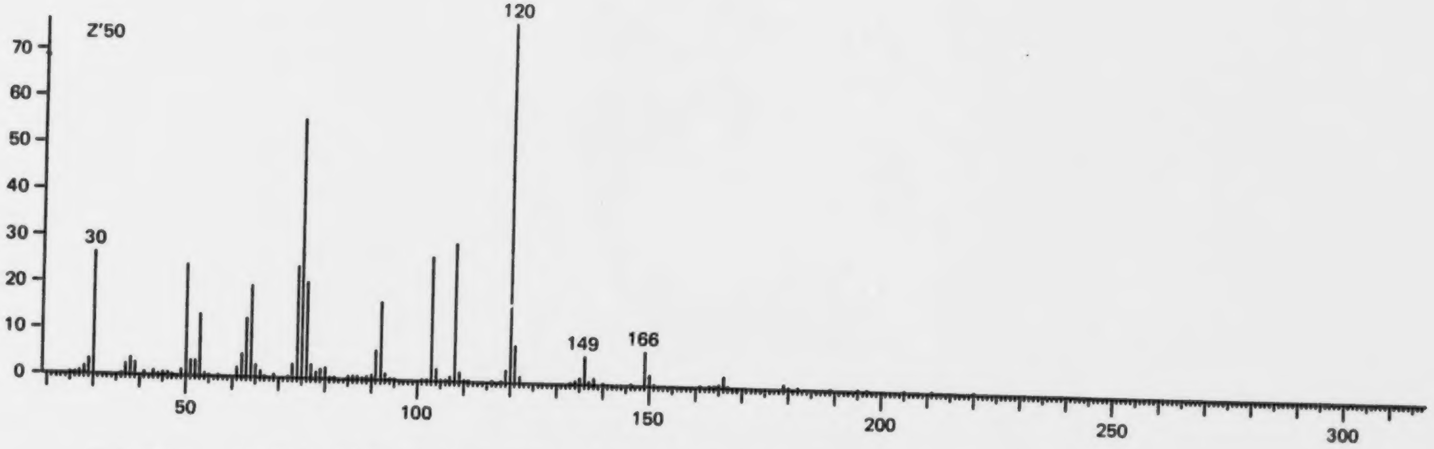


FIGURE 4 MASS SPECTRUM OF PRODUCT 1 FROM THE OZONATION OF CONDENSATE WATER — MW 166

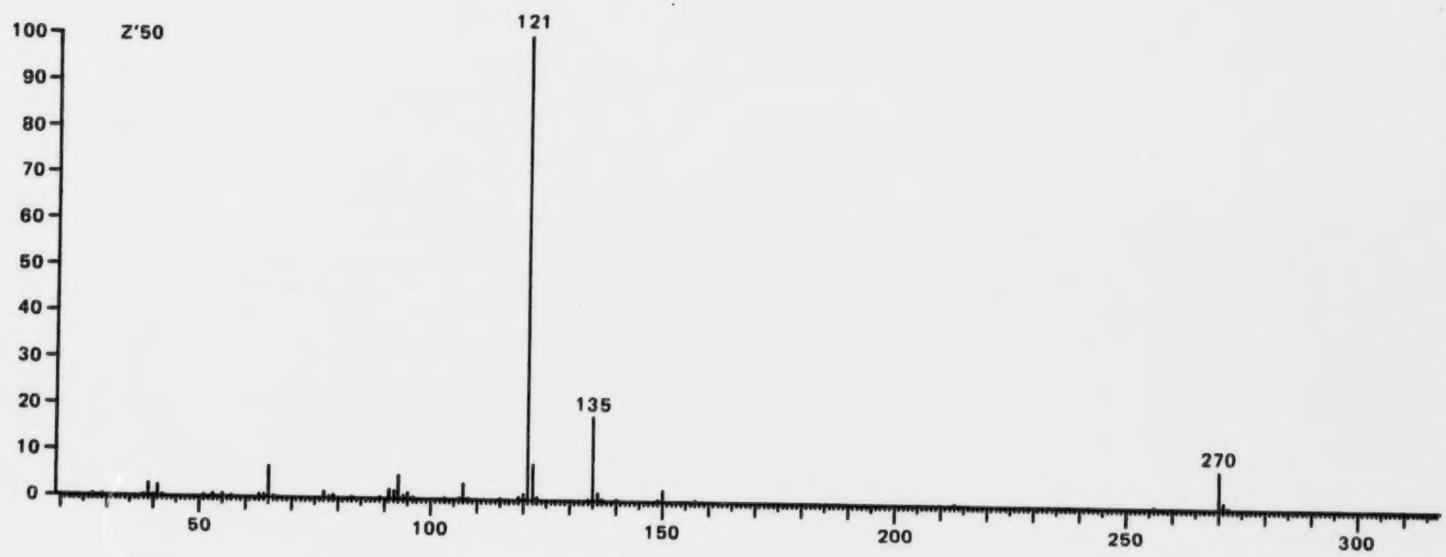


FIGURE 5 MASS SPECTRUM OF PRODUCT 2 FROM THE OZONATION OF CONDENSATE WATER — MW 270

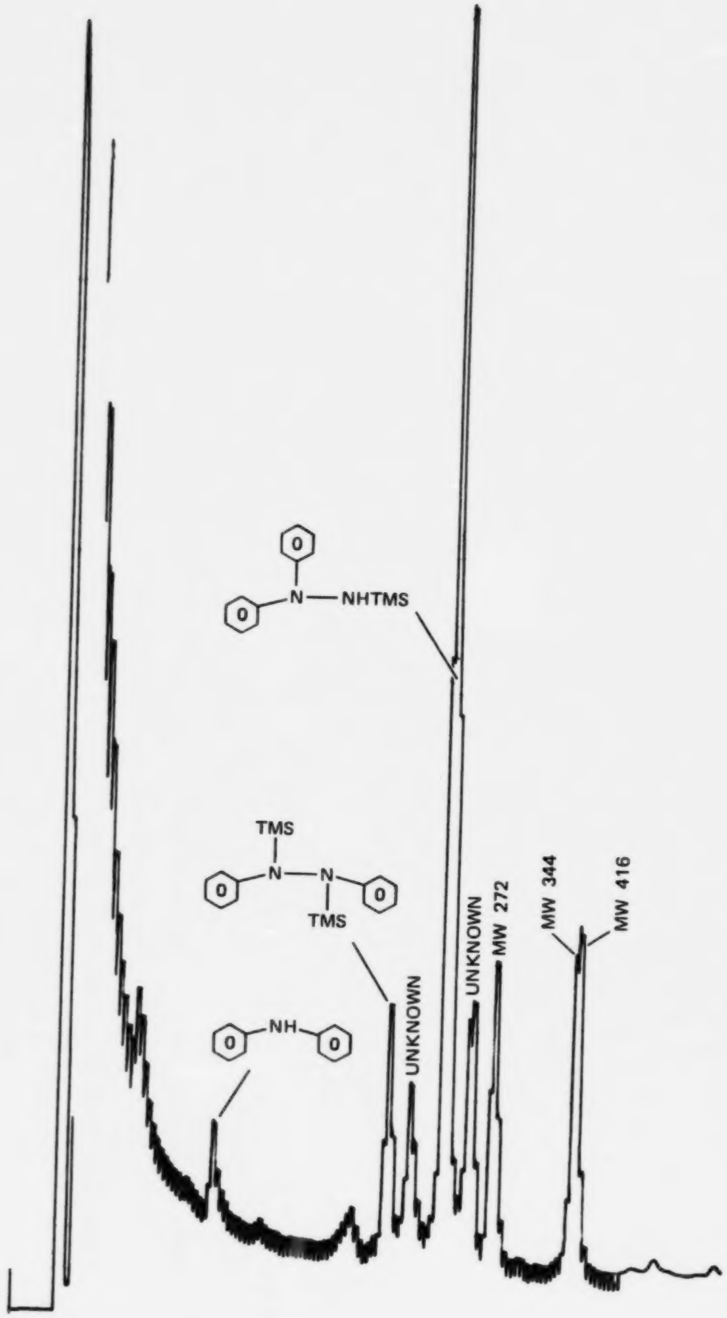


FIGURE 6 GAS CHROMATOGRAPHIC PROFILE OF OZONIZED 1, 1-DIPHENYHYDRAZINE — TMS

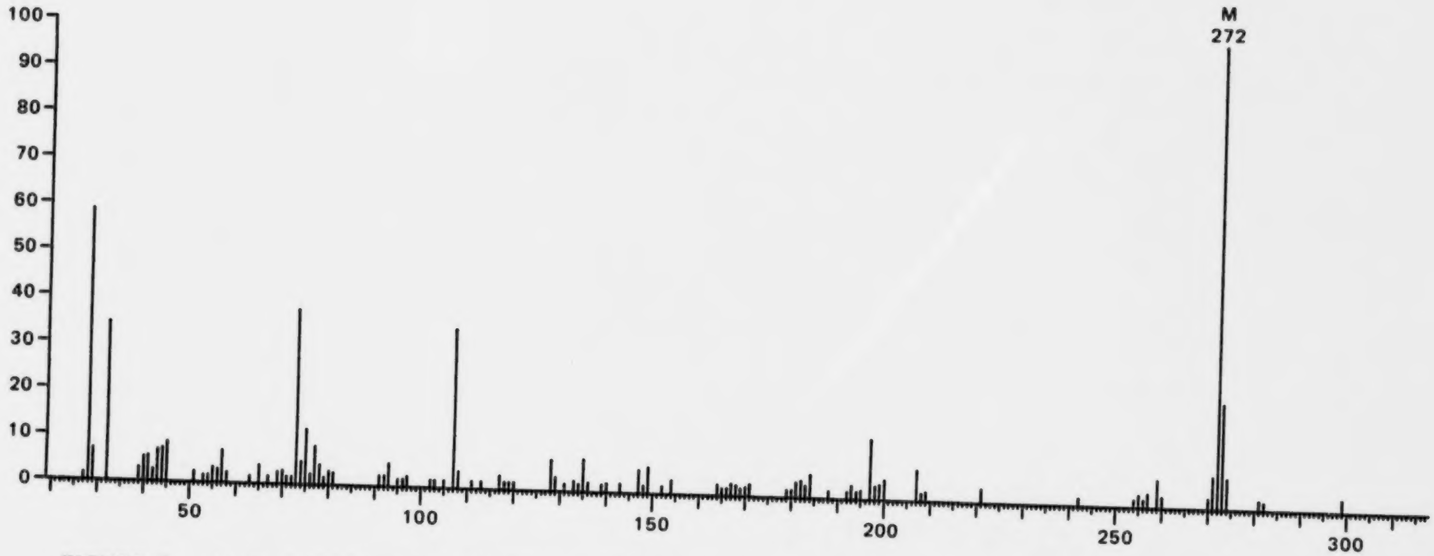


FIGURE 7 MASS SPECTRUM OF PRODUCT 1 FROM THE OZONATION OF 1,1-DIPHENYLHYDRAZINE — MW 272

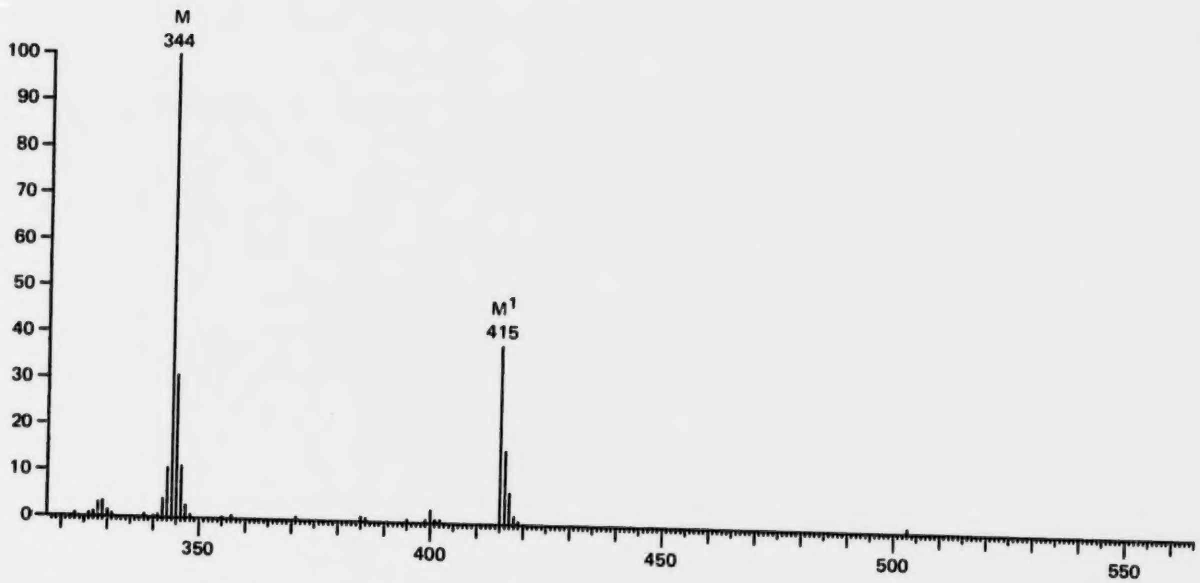


FIGURE 8 MASS SPECTRUM OF PRODUCT 2 FROM THE OZONATION OF 1,1-DIPHENYLHYDRAZINE

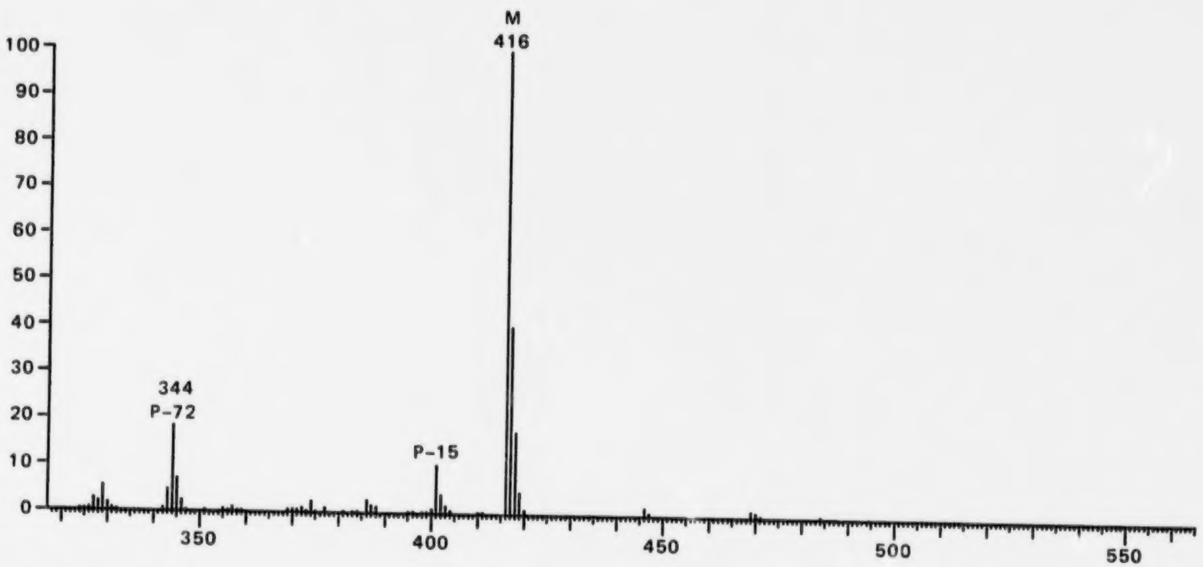
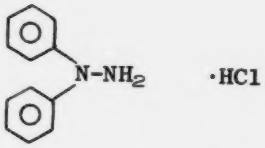


FIGURE 9 MASS SPECTRUM OF PRODUCT 3 FROM THE OZONATION OF 1,1-DIPHENYLHYDRAZINE — MW 416

1,1-Diphenylhydrazine

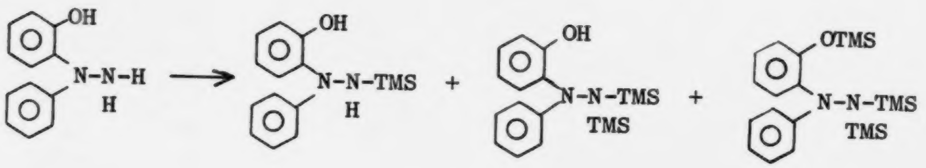


Source: Aldrich Chemical, Co.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH	
				Initial	Final
8190	6.7	2	34	2.9	2.2

The ozonation of 1,1-diphenylhydrazine proceeded rapidly. Diphenylamine, an impurity in 1,1-diphenylhydrazine, reacted slowly by comparison. One new product was formed but was not identified.

Figure 6 is the gc profile of the reacted solution. Silylation of the extract showed three new components in the chromatogram. Evaluation by gc/ms showed these peaks to be mon-, di-, and tri-silyl derivatives of a compound 16 mass units greater than the parent compound. Figures 7, 8, and 9 are the spectra for these products. A possible structure for this component may be represented as III in Eq. (6).

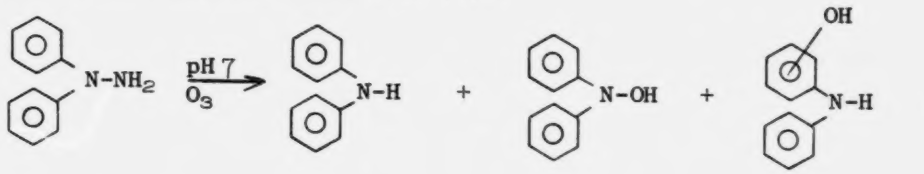


(6)

III

Ring hydroxylation is not unusual at the pH of the reaction mixture (2.9). The primary amine function is protonated at this pH and is probably nonreactive. Under buffered conditions (pH 7), both ring-

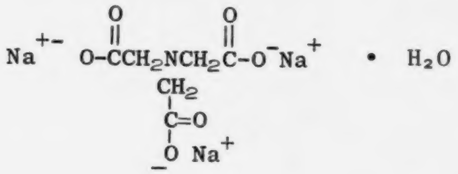
and nitrogen-hydroxylated derivatives of diphenylamine were found along with diphenylamine [Eq. (7)].



(7)

At each initial pH level, no diphenylnitrosamine was detected.

Nitrilotriacetic Acid, Trisodium Salt Monohydrate ($NTANa_3 \cdot H_2O$)



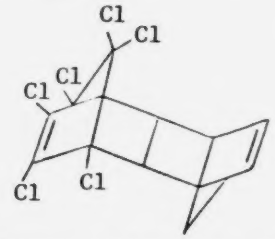
$$Na^{+-}O{-}\overset{O}{\overset{\|}{C}}CH_2NCH_2\overset{O}{\overset{\|}{C}}{-}O^{-}Na^{+} \cdot H_2O \quad (N{-}CH_2{-}C(=O){-}O^{-}Na^{+})$$

Source: Hampshire Chemical Corporation

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final	TOC Initial	TOC Final
10,450	5.7	60	50	110	9.7	4410	4410

$NTANa_3$ reacted with ozone and was monitored by uv. We did not attempt to identify new products. Also, no TOC reduction was noted.

Aldrin

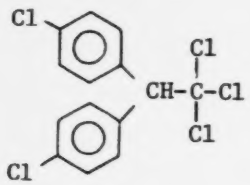


Source: Polyscience Corp.

Initial Conc.	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
100 mg suspended in 2 liters of H_2O	5.7	30	100	5.7	4.4

Since aldrin has very low solubility in water, it was ozonized as a colloidal suspension. No chemical investigation of reaction products was made.

DDT

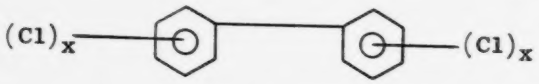


Source: Montrose Chemical Co.

Initial Conc. (ppb)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
7	5.7	90	86	7.9	4.5

Because of the low concentration of DDT in water, only low levels of DDT were ozonized. No new products were identified.

Aroclor 1254

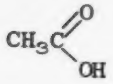


Source: Monsanto Co.

Initial Conc.	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
100 mg suspended in 2 liters of H_2O	5.7	60	--	6.7	4.5

Because of the low solubility of Aroclor 1254 in water, ozonations were performed on suspensions of 100 mg in 2 liters of water. No products were identified.

Acetic Acid

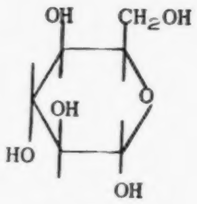


Source: Mallinckrodt Chemical Works

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (hr)	Amount Reacted (%)	pH Initial	pH Final
12,630	6.2	6	3.5	2.7	2.7

Acetic acid was found to be very unreactive with ozone. Only a slight decrease in acetic acid concentration was noted after 6 hr of ozonation. No new products were identified.

Glucose



Source: Merck & Co., Inc.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final	TOC Initial	TOC Final
10,000	4.2	15	34	7.2	3.6	4410	4410

Glucose was ozonized at an initial level of 10,000 ppm. However, no chromatographic data were acquired for the final solution. No TOC reduction was noted.

Urea

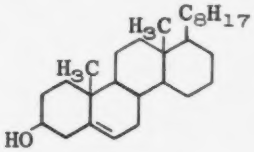
$$NH_2\overset{\overset{\displaystyle O}{\|}}{C}NH_2$$

Source: J. T. Baker Co.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
9432	4.1	180	3.8	7.6	4.2

Urea was unreactive with ozone, and no new products were identified. Urea was determined by gc as the TMS derivative.

Cholesterol

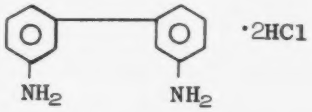


Source: ICN Pharmaceuticals, Inc.

Initial Conc.	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
Saturated	4.5	30	--	6.5	4.6

The water solubility of cholesterol was very low, and initial and final concentrations were not determined. Preparation of the initial solution was by stirring cholesterol in water at 40° for 2 hr, followed by filtration.

Benzidine·2HCl

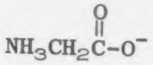


Source: ICN Pharmaceuticals, Inc.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
4830	4.5	30	64	2.8	2.1

Benzidine was found to be reactive with ozone (the solution turned dark). However, no new products were observed in the gc profile nor were product identifications made.

Glycine



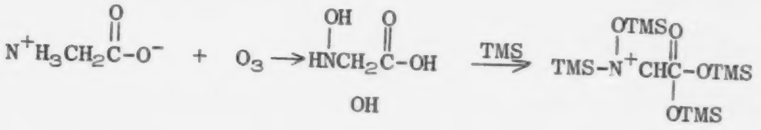
$$NH_3CH_2\overset{O}{\overset{\|}{C}}-O^-$$

Source: California Foundation for Biochemical Research

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
11,900	4.5	130	22	6.3	3.9

Glycine reacted slowly with ozone, and one new product was observed in the gc profile. No products were identified.

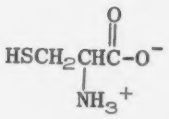
Glycine was gas chromatographed as the trimethylsilyl derivative, as shown in Figure 10. The gc/ms of the new product, m/e = 395, conforms with structure IV, shown in Eq. (8).



$$N^+H_3CH_2\overset{O}{\overset{\|}{C}}-O^- + O_3 \rightarrow \underset{OH}{\overset{OH}{HN}}CH_2\overset{O}{\overset{\|}{C}}-OH \xrightarrow{TMS} TMS-\overset{OTMS}{N^+}\underset{OTMS}{CH}\overset{O}{\overset{\|}{C}}-OTMS \qquad (8)$$

IV

Cysteine



$$HSCH_2\underset{NH_3^+}{CH}\overset{O}{\overset{\|}{C}}-O^-$$

Source: National Biochemical Corp.

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH	
				Initial	Final
9,622	4.5	32	17.6	5.0	2.8

Cysteine reacted slowly with ozone, and no new products were observed in the gc profile of the TMS derivatives.

Benzene

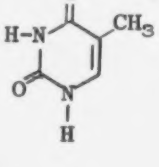
Source: Mallincrodt Chemical Works

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH	
				Initial	Final
510	5.2	25	~ 26	7.1	3.6

Benzene was found to be degassed readily from the reaction vessel. We determined the rate of loss by bubbling oxygen through the reactor and monitoring the benzene concentration by gc. As shown in Figure 11, the rate of loss, calculated from the slope of the curve, was approximately 280 ppm/min.

Then benzene was added dropwise to the reactor at a rate of about 1 ml/min, and the benzene concentration was monitored for 2 hr; 25 min of ozonation followed. No new products were observed in the gc profile, which is presented in Figure 12.

Thymine



Source: ICN Pharmaceuticals, Inc.

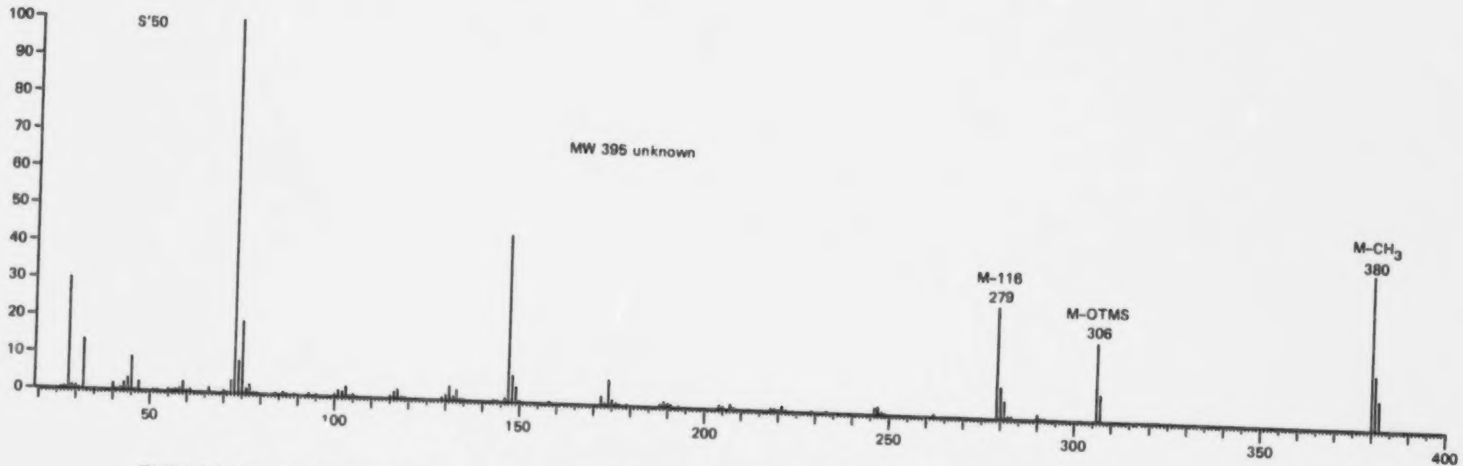


FIGURE 10 MASS SPECTRUM OF PRODUCT FROM THE OZONATION OF GLYCINE — TMS DERIVATIVE

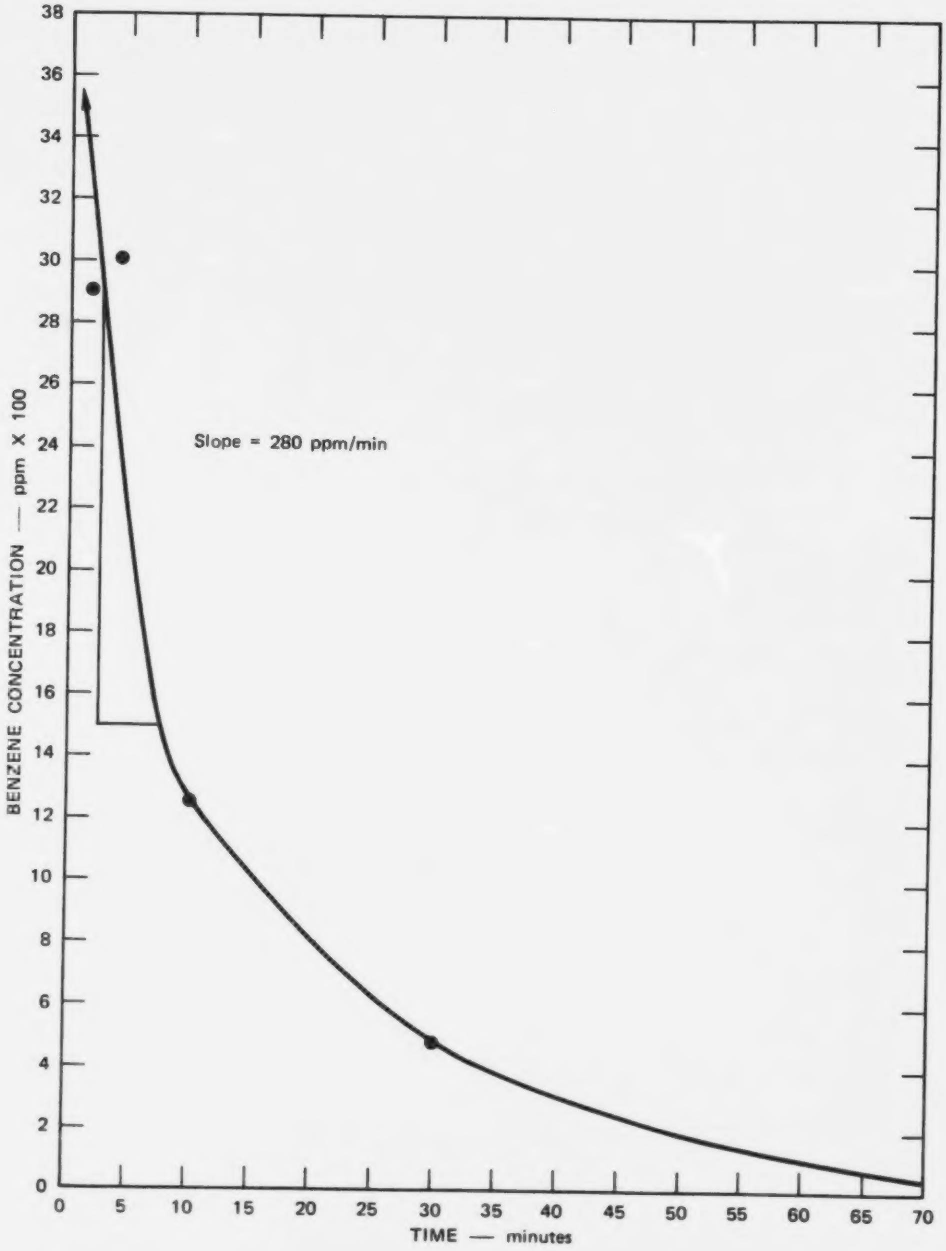


FIGURE 11 EVAPORATION RATE OF BENZENE FROM OZONATION VESSEL

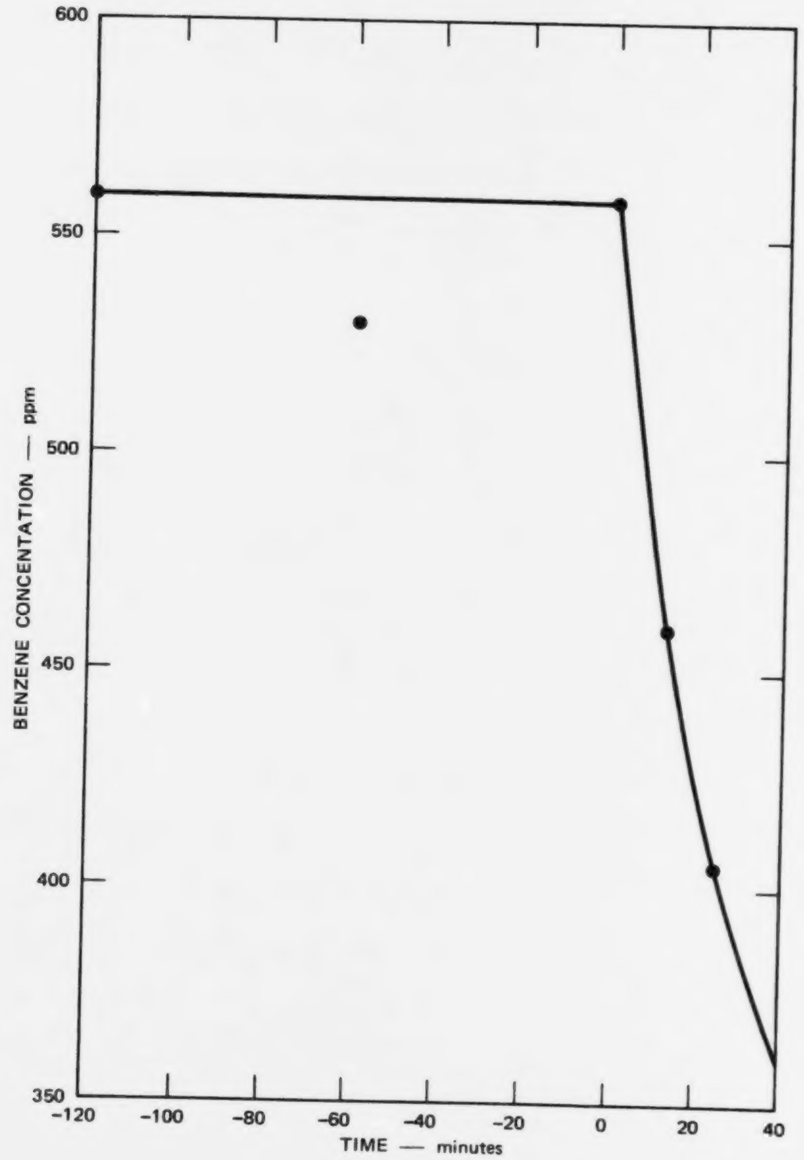
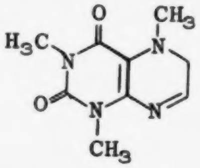


FIGURE 12 RATE OF OZONATION OF BENZENE (COMPENSATED FOR EVAPORATION)

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final	TOC Initial	TOC Final
2,227	5.2	16	94.6	7.3	3.0	1050	903

Thymine reacted readily with ozone, but no new components were observed in the gc profile after derivatization (trimethylsilylation). A slight TOC reduction was noted.

Caffeine

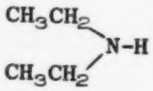


Source: Eastman Kodak

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
9,660	6.2	60	61	7.1	4.9

A 61% reduction in caffeine concentration was achieved in 60 min. The gc profile of the reacted solution showed two components--refer to Figure 13--with molecular ions corresponding to 142 and 210; Figures 14 and 15 are the gc profiles of these two products. Product 2 corresponds to the addition of an oxygen atom to caffeine. These products were not identified.

Diethylamine



Source: Matheson, Coleman & Bell

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH	
				Initial	Final
8,040	6.2	60	36.5	11.1	11.1

In the reaction of diethylamine with ozone, one product was identified as acetaldoxime (V). Confirmation of this structure was

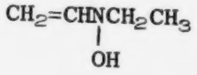
$$CH_3CH=N-OH$$

(V)

achieved through the synthesis of an authentic sample and by gc/ms comparison.

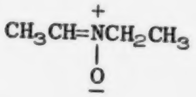
Another product was found with a molecular ion at m/e = 87 as shown in Figure 16. N-ethylacetamide, a potential reaction product, was eliminated as the product on the basis of comparative mass spectra.

The product may be represented as Compound VII or Compound VIII.



$CH_2=CH\underset{\underset{OH}{|}}{N}CH_2CH_3$

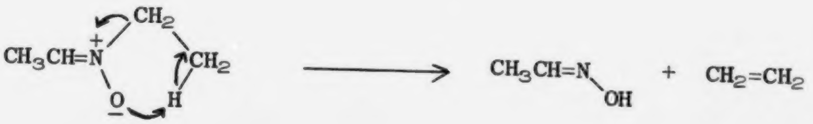
VII



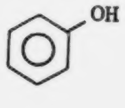
$CH_3CH=\overset{+}{\underset{\underset{\underline{O}}{|}}{N}}CH_2CH_3$

VIII

Compound VIII and VII possibly could be an intermediate to acetaldoxime [Eq. (9)] through an intramolecular elimination of ethylene.


(9)

Phenol



Source: Mallinckrodt Chemical Works

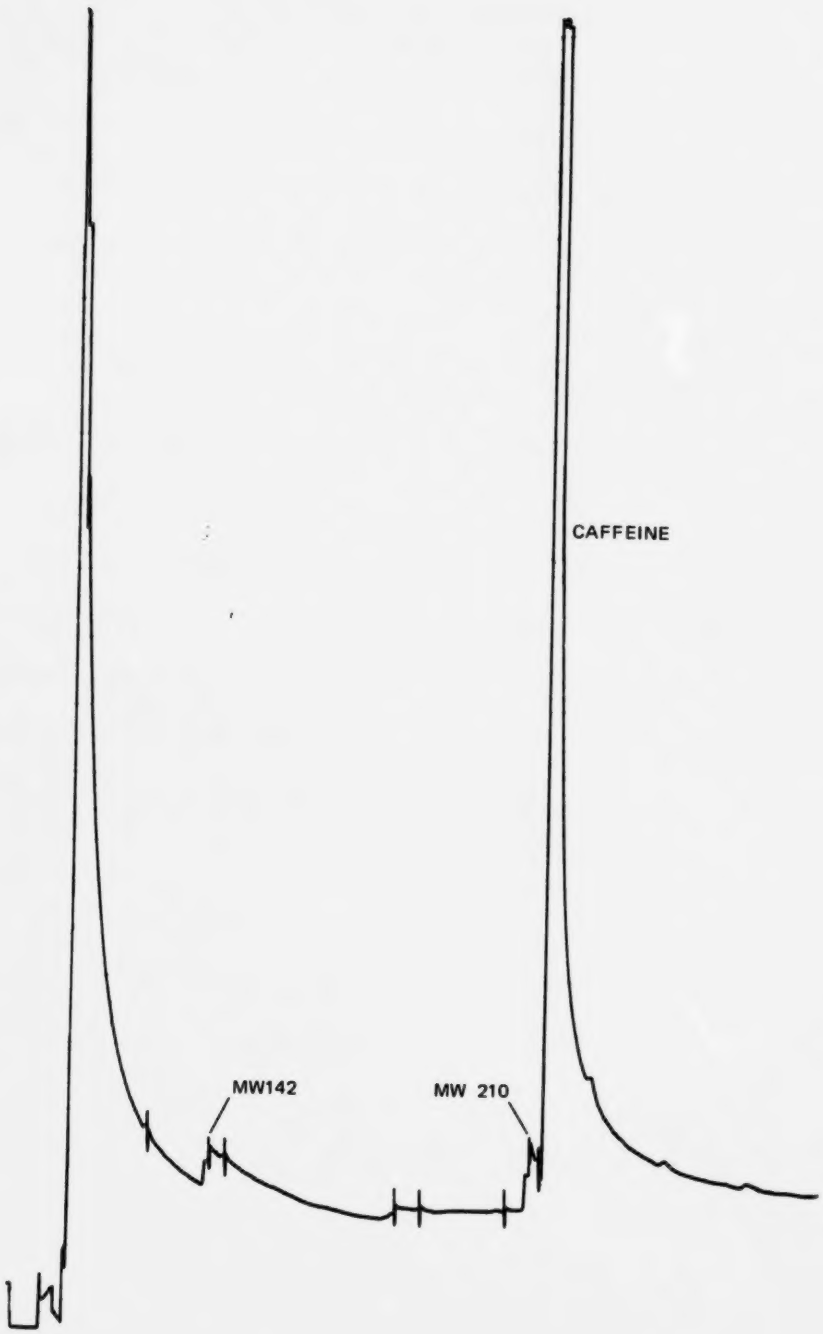


FIGURE 13 GAS CHROMATOGRAPHIC PROFILE OF OZONATED CAFFEINE

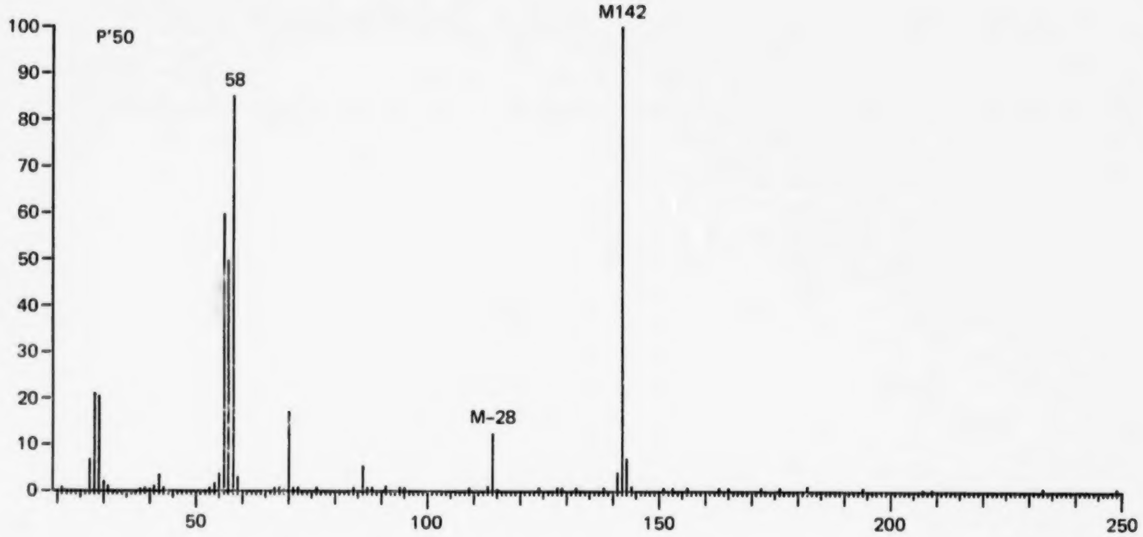


FIGURE 14 MASS SPECTRUM OF PRODUCT 1 FROM THE OZONATION OF CAFFEINE

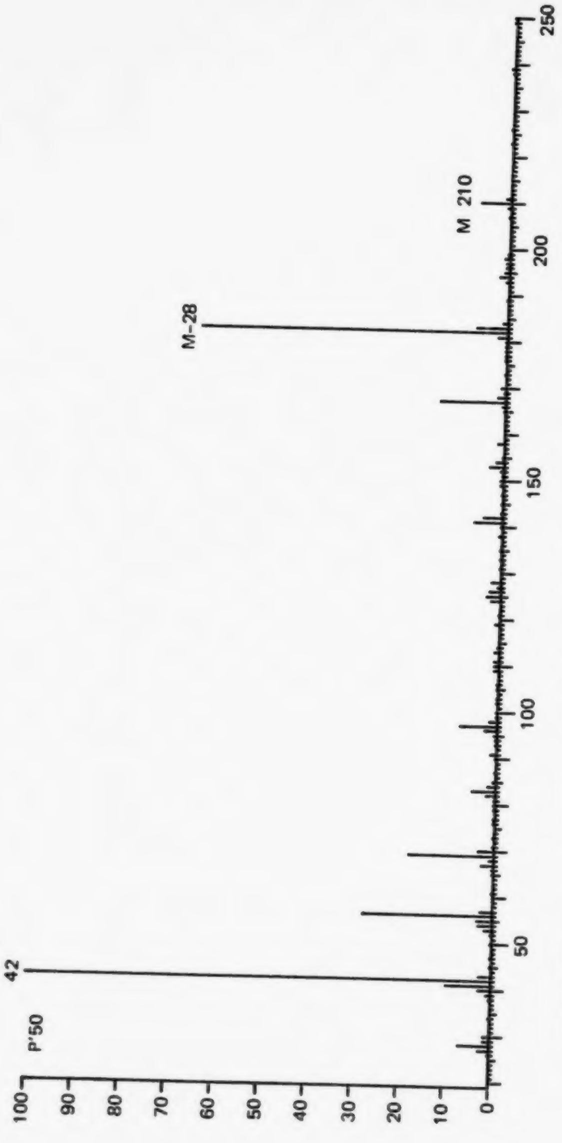


FIGURE 15 MASS SPECTRUM OF PRODUCT 2 FROM THE OZONATION OF CAFFEINE

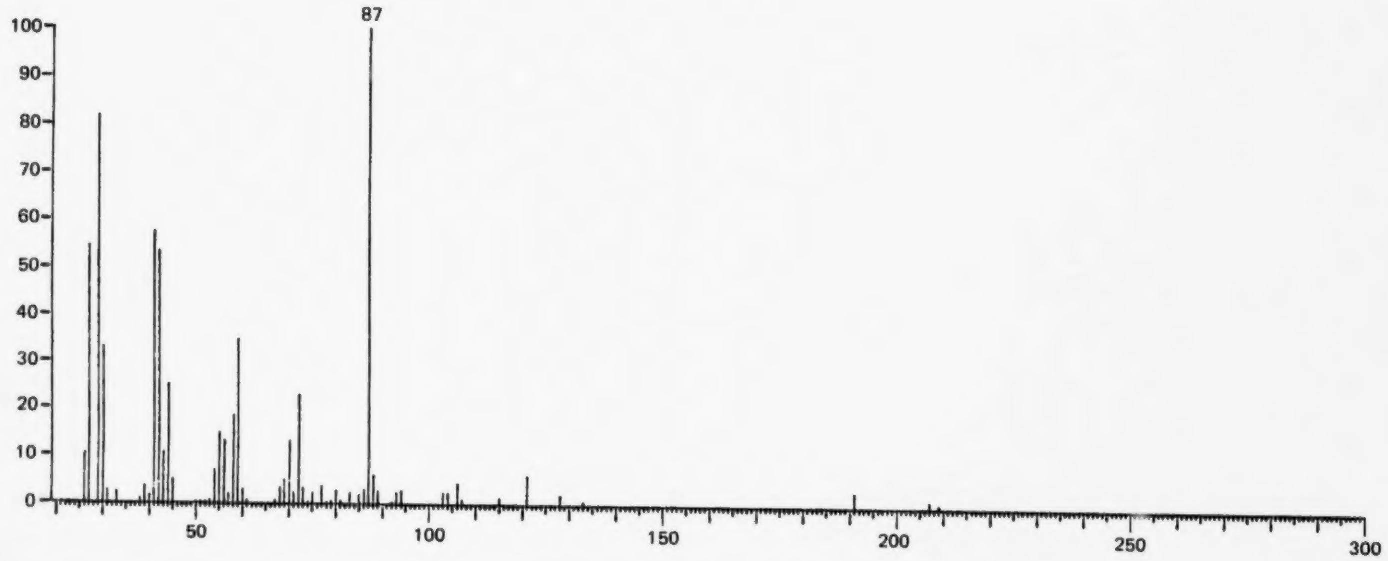
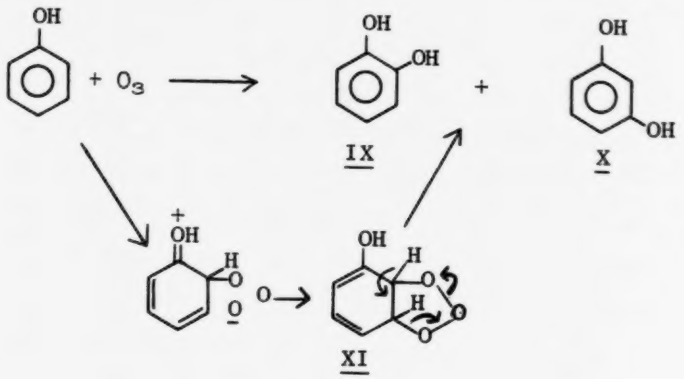


FIGURE 16 MASS SPECTRUM OF PRODUCT RESULTING FROM THE OZONATION OF DIETHYLAMINE

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH		TOC	
				Initial	Final	Initial	Final
9,140	3.8	110	41.1	6.9	2.4	6300	6300

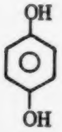
Phenol produced two products on ozonation that were identified as catechol (IX) and resorcinol (X), shown in Eq. (10).



(10)

Catechol has been observed by other workers,[13] but resorcinol has not. An intermediate such as XI may be responsible for both products. Compound XI could be formed initially by an electrophilic substitution of ozone on phenol, followed by nucleophilic substitution on the ring. Two modes for the elimination of oxygen are possible to yield both catechol and resorcinol.

Hydroquinone

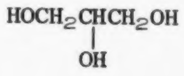


Source: Eastman Kodak

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH		TOC	
				Initial	Final	Initial	Final
15,260	3.8	95	15.5	6.9	3.2	5145	5040

Hydroquinone produced a dark-brown solution immediately after contact with ozone. However, no products except hydroquinone were visible in the gc profile of the reacted solution. Only a slight TOC reduction was observed.

Glycerol



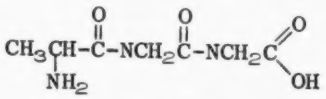
$$\mathrm{HOCH_2\underset{\underset{OH}{|}}{C}HCH_2OH}$$

Source: Matheson, Coleman & Bell

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
11,780	6.4	30	7.5	5.2	3.4
7,240	3.9	300	100	5.1	2.8

The ozonation of glycerol produced a component that oxidized iodide to iodine. No new components were observed by gc; however, 1,3-dihydroxyacetone was identified by gc/ms and was found to be an impurity in glycerol. A low-temperature titration of the ozonized solution with ceric sulfate indicated the presence of an oxidant at 1.5×10^{-3} N.

Alanyl glycyl glycine



$$\mathrm{CH_3\underset{\underset{NH_2}{|}}{C}H-\overset{\overset{O}{\|}}{C}-NCH_2\overset{\overset{O}{\|}}{C}-NCH_2C\begin{matrix}\nearrow O\\ \searrow OH\end{matrix}}$$

Source: ICN Pharmaceuticals

Initial Conc. (ppm)	Ozone Conc. (g/hr)	Reaction Time (Min.)	Amount Reacted (%)	pH Initial	pH Final
462	6.4	45	0	5.3	3.8

No reaction of this tripeptide was observed with ozone.

Microbiological Mutagenicity Assays

Table 1 summarizes the mutagenicity results for testing 29 compounds. The actual sets of data are appended. Table 2 presents typical results for mutagenic positive controls for the *Salmonella* and the *Saccharamyces* assays. These known mutagens were assayed when each chemical was tested, but the results are not included with each chemical in the tables. Table 140 presents the μmoles of compound added to the highest nontoxic dose tested in the Salmonella assay.

2,4-Dinitrotoluene

At the concentrations tested (9.0 μg/plate for the highest dose), 2,4-DNT was not mutagenic in the *S. typhimurium* before or after ozonation. The highest concentration tested in the *S. cerevisiae* assay, 0.001%, was neither mutagenic nor toxic. Generally, a greater number of mitotic recombinants was observed after ozonation, but the response was not dose related.

Benzoic Acid

Benzoic acid was not mutagenic in either assay, before or after ozonation. The highest doses tested were 9.25 μg/plate in the *S. typhimurium* assay and .05% in the *S. cerevisiae* assay.

Ethanol

Before ozonation, ethanol was not mutagenic. After prolonged ozonation (4 to 6 hours), mutagenic activity was observed. The number of revertants in strain TA100, increased slightly (about 2-fold), and a greater than 20-fold increase occurred in the number of mitotic recombinants in *S. cerevisiae*. Metabolic activation significantly decreased mutagenic activity. Acetaldehyde was considered as the possible mutagen, but when tested with *S. cerevisiae* it was not mutagenic. Preliminary experiments indicated that hydrogen peroxide was weakly mutagenic and toxic in TA100 and in *S. cerevisiae*. However, the mutagenic activity we observed with H_2O_2 was far less (1/100) than expected if it was the mutagen in ozonated ethanol. Ethyl hydroperoxide, with the appropriate oxidizing potential, was mutagenic but far more toxic than ozonated ethanol. A mixture of H_2O_2, acetaldehyde, ethanol, and acetic acid in proportions approximating their concentrations after extended ozonation of ethanol had approximately the same mutagenic activity as ozonated ethanol but was quite toxic.

In another reconstruction experiment, acetaldehyde and H_2O_2 were added to form ethylhdyroxy hydroperoxide, which was mutagenic but more toxic than ozonated ethanol.

A mixture of acetaldehyde, formaldehyde, and H_2O_2 gave a product identical to ozonized ethanol in mutagenic activity and in nmr spectrum.

Benzo(a)pyrene

The known carcinogen/mutagen (when metabolically activated), benzo(a)pyrene was not mutagenic in the presence or in the absence of metabolic activation. However, the concentration before ozonation used in these assays (1.3×10^{-3} μg/plate) was considerably lower than the amount required to demonstrate mutagenicity in the *S. typhimurium* assay (1 to 5 μg/plate). This limitation is due to the solubility of benzo(a)pyrene in water for ozonation experiments. If mutagenic products were formed (all detectable benzo(a)pyrene was destroyed by ozonation), their concentration was insufficient for chemical detection and for mutagenic activity.

HMX

HMX was not mutagenic before or after ozonation in these assays.

RDX

RDX was not mutagenic before or after ozonation in these assays.

Oleic Acid

Oleic acid and its ozonation products were not mutagenic in these assays.

Humic Acids

The humic acids and products formed from them by ozonation were not mutagenic in these assays.

Condensate Water

The TNT condensate water appeared to be mutagenic in assays with *S. typhimurium* but not with *S. cerevisiae* before ozonation. After ozonation, the TNT condensate water was weakly mutagenic on strains TA1535 and TA100 in the absence of metabolic activation in one experiment. Thus, the mutagenic activity of this compound is virtually lost when the TNT condensate water is ozonated.

1,1-Diphenylhydrazine

Before ozonation, diphenylhydrazine (2.048 mg/plate) was toxic but was not mutagenic in the *S. typhimurium* assays. After ozonation, it was weakly mutagenic in *S. typhimurium* in one experiment, but it was not mutagenic in a second experiment. The strains, which were mutated were TA1535 and TA100 (although the data are not shown, the weak mutagenic activity in this experiment was also observed in *Escherichia coli* WP2). Diphenylhydrazine was not mutagenic in assays with *S. cerevisiae*.

Nitrilotriacetic Acid, Trisodium Salt

$NTANa_3$ was not mutagenic in *S. typhimurium* before or after ozonation. In *S. cerevisiae*, an elevation of the number of mitotic recombinants was noted with the two highest doses tested with metabolic activation after ozonation.

Aldrin, DDT, Aroclor 1254, Acetic acid, Glucose, Urea, and Cholestorol

These compounds were not mutagenic in these assays before or after ozonation.

Benzidine

The known carcinogen/mutagen (when metabolically activated) benzidine was mutagenic in *S. typhimurium* when metabolically activated before ozonation (1.25 mg/plate). Mutagenic activity was observed both in the presence and in the absence of metabolic activation after ozonation. The result suggests that oxidation of the amine group (e.g., to a hydroxylamino or nitro group) may have occurred as a result of ozonation.

Before ozonation, benzidine was not mutagenic in *S. cerevisiae*, even in the presence of a metabolic activation system. However, after ozonation, mutagenic activity was observed. These results reinforce the observations in *S. typhimurium* that direct-acting mutagenic products are formed when benzidine is ozonated.

Glycine, Cysteine, Benzene, Thymine, Caffeine, and Diethylamine

These compounds were not mutagenic before or after ozonation.

Phenol

Phenol was not mutagenic before ozonation. After ozonation, toxic products were present and the solution was mutagenic in *S. cerevisiae* but not in *S. typhimurium*.

Hydroquinone

Like phenol, hydroquinone was not mutagenic before ozonation, but the products were mutagenic in *S. cerevisiae* (only at the highest dose tested) but not in *S. typhimurium*.

Glycerol

Before ozonation, glycerol was not mutagenic in either assay. Mutagenic activity in *S. typhimurium* strains TA100 and TA98 and in *S. cerevisiae* was observed when glycerol was ozonated for an extended time (5 hours). However, when ozonated for 0.5 hour and 1 hour, mutagenesis was not observed.

Alanylglycylglycine

Alanylglycylglycine was not mutagenic in either assay, before or after ozonation.

Samples of water submitted by Mr. G. Michael DeGraeve of the Wyoming Bioassay Laboratory were tested for mutagenicity. The duplicate samples tested were untreated, chlorinated, chlorinated-dechlorinated, chlorobrominated, and ozonated water. None of these samples was mutagenic.

CONCLUSIONS

Ozonation of alcohols (ethanol) produced mutagenic activity that can be associated with hydroperoxide formation. Because ethanol is a common industrial and household chemical, hydroperoxides may be of significant environmental concern.

Ozonation can produce intermediates that bypass the need for metabolic activation in biological systems. The overall effect of this ozonation activation is difficult to assess. It is possible that the active products formed could react with other organics either before being consumed or when they reach the stomach or intestine. Thus, their effective level would be reduced. A more thorough study using animals would be necessary to determine whether the hazard of such materials (e.g., aromatic amines) would be altered by ozonation.

These facts do not preclude the use of ozone as a water purification technique. They only suggest that the technique is not perfectly clean. The extent to which the above reactions occur in the environment may be undetectable or insignificant to be of concern. However, under our laboratory conditions, the potential exists for ozone to produce intermediate products possessing mutagenic activity.

The results of these experiments are not completely indicative of what might occur in a water treatment facility. The experiments were designed with the oxidant as the limiting reagent to ensure that intermediate products would be available during biological screening. In a water treatment facility, intermediate products can be completely oxidized if the oxidant-to-substrate ratio is high. Also, at low substrate concentrations bimolecular reactions between substrate intermediates become less probable, thus affecting both products and distribution of products. Therefore, because these experiments were performed at high substrate concentrations and low oxidant-to-substrate ratios, the

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results may be misleading when applied to a water treatment facility situation. The results may have more significance at facilities where substrate concentrations are high, such as those that might be found in an industrial effluent.

The sensitivity of the biological system necessitates the use of extreme conditions and high substrate levels to achieve significant concentrations of intermediate products in water. The mutagenic screening of environmental levels of organics could be accomplished by using extraction techniques to form a concentrate. However, the chemical transformations caused by ozone usually involve the incorporation of oxygen, which tends to make the intermediate products more polar than the initial substrate so that these products have greater water solubility. Thus, by using extraction techniques, polar intermediates may not be efficiently removed and concentrated. For example, with ethanol the mutagen (hydroperoxide) would not partition to an organic phase and would have been missed in the bioassay if extraction techniques had been used.

These results indicate that microbial mutagenicity assays are useful for the rapid and relatively inexpensive analysis of potentially mutagenic and/or carcinogenic organic materials. In this study, a number of mutagenic products were identified in solutions treated with ozone. In particular, when ethanol was ozonized, a product(s) was formed that increased mitotic recombination in *S. cerevisiae*. The product(s) was not identified in the routine chemical screening. A considerable number of reconstruction experiments were necessary using *S. cerevisiae* to monitor the biological activity before a dihydroperoxide was identified as the mutagenic agent. Also, when benzidine was ozonized, a product(s) was formed that differed from the parent compound in that it no longer required metabolic activation for mutagenic activity in *S. typhimurium*. However, no chemical product(s) was identified. Therefore, the microbial mutagenicity assays clearly can contribute significantly to the evaluation of the potential hazards of alternative disinfection procedures. Nevertheless, the problems in extrapolating the results from such procedures to assessment of risk are significant.

In the absence of a complete material balance of the reacted substrate and the screening of the individual intermediate products, the microbial mutagenic assays serve as a useful prescreen to identify systems with potential biological activity and serve to indicate those systems in which further detailed investigations are necessary.

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APPENDIX A

This appendix is included to supplement the description of the general analytical methods that were given on pages 7 and 8 and detail the specific experimental conditions employed for each chemical studied.

Urea

Gas chromatographed as TMS derivative (BSTFA)

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on
Gas Chrom Q (80-100)

Temperature - 125° C

Flow rate - 30 ml/ min N_2

Retention time - 4.92 min (urea, tetra-TMS derivative)
9.53 min (1,3-dinitrobenzene, internal standard)

Detection - flame ionization

Acetic Acid

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on
Gas Chrom Q

Temperature - 125° C

Flow rate - 30 ml/min N_2

Retention time - 0.44 min (acetic acid)
1.81 min (phenol, internal standard)

1,1-Diphenylhydrazine

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on
Gas Chrom Q

Temperature - 200 → 220° at 4°/min

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Flow rate - 30 ml/min N_2

Retention time - 5.63 (1,1-diphenylhydrazine as Di-TMS derivative)
6.94 (caffeine, internal standard)

Condensate Water and 2,4-Dinitrotoluene

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Gas Chrom Q

Temperature - 150 → 225° at 8°/min

Flow rate - 30 ml/min

Retention time - 6.77 min (2,4-dinitrotoluene, major component)
7.45 min (2,4,6-trinitrotoluene, internal standard)

Detection - flame ionization

Benzene

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Gas Chrom Q

Temperature - 80 → 200° at 8°/min, 3 min hold

Flow rate - 30 ml/min

Retention time - 0.52 min (benzene, external standard quantitation)

Benzoic Acid

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Gas Chrom Q

Temperature - 150 → 220° at 6°/min

Flow rate - 30 ml/min N_2

Retention time - 3.61 min (benzoic acid)
8.75 min (1,3-dinitrobenzene, internal standard)

Detection - flame ionization

Hydroquinone

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Gas Chrom Q

Temperature - 170 → 220° at 8°/min

Flow rate - 30 ml/min N_2

Retention time - 1.29 (phenol, internal standard)
3.55 (hydroquinone)

Detection - flame ionization

Diethylamine

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 100 → 230° at 15°/min

Flow rate - 12 ml/min N_2

Retention time - 1.31 min (diethylamine)
18.93 min (caffeine, internal standard)

Detection - flame ionization

Caffeine

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 150 → 220 at 8°/min

Flow rate - 30 ml/min N_2

Retention time - 1.22 min (phenol, internal standard)
12.01 min (caffeine)

Detection - flame ionization

Thymine

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 150 → 220° at 4°/min

Flow rate - 28 ml/min

Retention time - 7.51 min (thymine, TMS derivative)
9.66 min (1,3-dinitrobenzene)

Detection - flame ionization

Cysteine

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 170 → 220° at 8°/min

Flow rate - 30 ml/min N_2

Retention time - 5.81 min (2,4-dinitrotoluene, internal standard)
7.16 min (cysteine, TMS derivative)

Detection - flame ionization

Glycine

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 100 → 220° at 10°/min

Flow rate - 30 ml/min N_2

Retention time - 4.63 min (glycine, TMS derivative
10.61 min (2,4-dinitrotoluene, internal standard)

Detection - flame ionization

Benzidine•2 HCl

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 200 → 220° at 8°/min

Flow rate - 30 ml/min N_2

Retention time - 2.84 min (1,3-dinitrobenzene, internal standard)
12.75 min (benzidine, TMS derivative)

Detection - flame ionization

Nitrilotriacetic Acid, Trisodium Salt Monohydrate (NTA)

NTA was determined colorimetrically at 535 nm by the displacement of zirconium from a zirconium-xylenol orange complex [Versene NTA Chelating Agents, The Dow Chemical Co., p. 26 (1967)].

DDT

Column - 1.8 m x 2 mm glass column packed with 3% OV-17 on Gas Chrom Q

Temperature - 200° C

Flow rate - 40 ml/min N_2

Retention time - 5.52 min (dieldrin, internal standard)
10.81 min (DDT)

Detection - electron capture

Aroclor 1254

Column - 1.8 m x 2 mm glass column packed with 10% DC-200 on Gas Chrom Q

Temperature - 200° C

Flow rate - 40 ml/min N_2

Retention time - 15.0 min (Aroclor, seventh peak of complex)

Detection - electron capture

Aldrin

Column - 1.8 m x 2 mm glass column packed with 3% OV-17 on Gas Chrom Q

Temperature - 170°

Flow rate - 40 ml/min

Retention time - 4.02 min (Aldrin)
8.34 min (Dieldrin, internal standard)

Detection - electron capture

RDX

Column - 4 mm ID x 30 cm μ Bondapak C_{18} (Waters Associates)

Solvent - methanol/water (79/21)

Flow rate - 1.6 ml/min

Detection - UV @ 254 nm

Retention time - 2.65 min (RDT)
5.78 min (Benzophenone, internal standard)

HMX

Column - 4 mm x 30 cm μ Bondapak C_{18} (Waters Associates)

Solvent - Methanol/water (79/21)

Flow rate - 1.6 ml/min

Detection - UV @ 254 nm

Retention time - 1.75 min (HMX)
5.78 min (Benzophenone, internal standard)

Benzo(a)pyrene

Column - 4 mm x 30 cm μ C_{18} (Waters Associates)

Solvent - Methanol/water (85/15)

Flow rate - 1.6 ml/min

Retention time - 6.67 min (Benzo(a)pyrene)

Detection - Fluorescence @ 360 nm

Phenol

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200
on Chromosorb WHP

Temperature - 75-210° @ 4°/min, 2 min hold

Flow rate - 30 ml/min N_2

Retention time - 6.5 min (phenol)
24.6 min (benzophenone, internal standard)

Detection - flame ionization

Glycerol

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200
on Chromosorb WHP

Temperature - 90 →230° @ 8°/min, 2 min hold

Flow rate - 20 ml/min N_2

Retention time - 5.4 min (glycerol, TMS derivative)

Detection - flame ionization

Glucose

Gas chromatographed as the TMS derivative

Column - 1.8 m x 2 mm glass column packed with 10% DC-200
on Chromosorb WHP

Temperature - 120-230° @ 8°/min

Retention time - 7.2 mm (glucose, TMS derivative)

Detection - flame ionization

Cholesterol

Column - 1.8 m x 2 mm glass column packed with 1% OV-17
on Chromosorb WHP

Temperature - 220 - 250° @ 4°/min

Flow rate - 30 ml/min N_2

Retention time - 13.08 min (Comprostanol, interal standard)
14.40 min (cholesterol)

Detection - flame ionization

Oleic Acid

Gas chromatographed as the TMS derivative

Column - 1.8 m x 4 mm glass column packed with 10% DC-200 on Chromosorb WHP

Temperature - 140 - 220° @ 4°/min

Flow rate - 30 ml/min N_2

Retention time - 8.6 min (oleic acid, TMS derivative)

Detection - flame ionization

Ethanol

Column - 1.8 m x 3 mm metal column packed with 10% DEGS on gas chrom Q

Temperature - 100°C

Flow rate - 20 ml/min N_2

Retention time - 1.77 min (ethanol)
2.55 min (n-propanol, internal standard)

Detection - flame ionization

APPENDIX

B

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
AND ESCHERICHIA COLI WP2

DINITROTOLUENE

EXPERIMENT 5

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	4	5	8	9	121	39
		+	2	16	11	6	105	32
Pre-Ozonation Dinitrotoluene	50	-	2	10	14	1	111	35
	50	+	1	12	9	4	136	37
Ozonation Dinitrotoluene	0.1	-	2	11	11	6	102	25
	0.2	-	2	10	4	6	79	32
	0.6	-	2	11	9	3	111	19
	1.2	-	1	3	6	5	99	49
	2.4	-	0	17	8	4	110	30
	6.0	-	3	18	9	6	125	36
	0.1	+	1	6	7	9	116	38
	0.2	+	3	11	11	5	97	27
	0.6	+	0	10	9	11	102	28
	1.2	+	1	7	10	7	115	36
	2.4	+	1	6	8	8	96	44
	6.0	+	1	9	18	5	117	45

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Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DINITROTOLUENE

EXPERIMENT 15

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	36	5	Contam.	25	117	62
		+	24	9	Contam.	35	112	98
Positive Control								
4-o-Tolylazo-o-toluidine	25	+			Contam.	105		
N-Methyl-N'-nitro-N-nitrosoguanidine	2	-	145		Contam.			190
81		-			Contam.		~1500	
9 AA	20	-		~2000	Contam.			
Pre-Ozonation Dinitrotoluene	20	-	Contam.	Contam. 26	Contam.	Contam.	Contam.	
	20	+	Contam.	Contam.	Contam.	Contam.	Contam.	
Ozonation Dinitrotoluene	0.2	-	60	4	Contam.	20	3	67
	0.4	-	37	5	Contam.	21	3	72
	0.9	-	44	7	Contam.	16	3	86
	1.8	-	36	6	Contam.	16	5	64
	3.6	-	42	3	Contam.	21	2	62
	9.0	-	59	4	Contam.	41	15	72
	0.2	+	34	4	Contam.	29	4	68
	0.4	+	14	9	Contam.	38	3	97
	0.9	+	20	3	Contam.	23	1	99
	1.8	+	25	9	Contam.	35	2	99
	3.6	+	21	3	Contam.	28	9	93
	9.0	+	34	7	Contam.	50	8	78

Table 3

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

BENZOIC ACID

EXPERIMENT 4

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					*E. coli*
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	25	16	7	15	134	33
		+	17	15	18	31	136	46
Pre-Ozonation Benzoic Acid	2500	-	156	15	10	13	125	16
	2500	+	10	15	8	20	99	23
Ozonation Benzoic Acid	19	-	102	10	17	17	43	67
	37	-	89	16	17	22	38	70
	93	-	121	15	15	20	29	51
	185	-	105	14	13	20	40	53
	370	-	110	18	16	20	42	72
	925	-	47	11	7	10	13	56
	19	+	18	23	23	22	55	58
	37	+	15	22	26	26	46	76
	93	+	19	12	26	20	43	80
	185	+	22	18	23	20	23	88
	370	+	16	17	25	27	44	98
	925	+	12	17	21	18	11	43

Table 4

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BENZOIC ACID

EXPERIMENT 15

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	36	5	Contam.	25	117	62
		+	24	9	Contam.	35	112	98
Positive Control								
4-o-Tolylazo-o-toluidine	25	+			Contam.	105		
N-Methyl-N'-nitro-N-nitrosoquanidine	2	-	145		Contam.			190
81		-						
9 AA	20	-		~2000	Contam.		~1500	
Pre-Ozonation	905	-	25	4	Contam.	20	45	63
Benzoic Acid	905	+	42	7	Contam.	30	84	67
Ozonation	16	-	36	6	Contam.	25	30	63
Benzoic Acid	31	-	58	3	Contam.	24	6	72
	78	-	39	3	Contam.	21	17	69
	156	-	36	9	Contam.	36	17	52
	311	-	32	4	Contam.	16	20	72
	778	-	32	1	Contam.	16	128	66
	16	+	24	10	Contam.	33	6	71
	31	+	21	5	Contam.	38	7	80
	78	+	42	3	Contam.	25	8	66
	156	+	21	5	Contam.	20	7	71
	311	+	32	3	Contam.	30	8	62
	778	+	30	5	Contam.	25	115	62

Table 5

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

ETHANOL

EXPERIMENT 1

Compound	Amount of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	6	16	18	28	99
		+	5	18	15	26	100
Pre-Ozonation Ethanol	1950 ug	-	37	30	31	36	112
	1950 ug	+	34	25	25	26	147
10-minute Ozonation* Ethanol	0.005	-	10	19	24	22	134
	0.01 ml	-	27	29	28	16	81
	0.025	-	20	24	25	25	110
	0.05	-	13	18	27	27	101
	0.1	-	11	34	27	49	95
	0.25	-	12	29	29	36	126
	0.005 ml	+	10	20	24	14	107
	0.01	+	10	12	28	22	87
	0.025	+	9	24	18	16	108
	0.05	+	12	22	20	12	102
	0.1	+	5	12	22	21	110
	0.25	+	5	16	14	20	126
4-hour Ozonation Ethanol	29 µg	-	18	27	25	15	94
	59	-	19	26	20	18	118
	146	-	7	12	28	18	101
	293	-	16	15	32	21	146
	585	-	8	21	7	toxic	173
	1463	-	3	toxic	toxic	toxic	toxic
	29 µg	+	7	13	23	22	133
	59	+	6	20	11	22	121
	146	+	9	19	11	29	114
	293	+	12	21	25	14	105
	585	+	5	Contam.	13	20	136
	1463	+	3	11	toxic	25	122

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*% reacted not quantitated.

Table 6

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

ETHANOL

EXPERIMENT 16

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	59	17	10	40	231
		+	45	18	24	54	263
Positive control							
9 AA	100	-		1000			
B-propiolactone	50	-	1000				
Biphenylamine	500	+			500		
AF_2	0.1	-				600	1200
Pre-Ozonation Ethanol	1940	-	105	78	94	107	262
	1940	+	142	85	113	123	299
Ozonation Ethanol	10	-	42	17	18	35	258
	21	-	33	15	14	66	238
	52	-	26	9	14	66	233
	105	-	17	2	9	34	258
	210	-	4	Toxic	Toxic	13	288
	524	-	0	2	Toxic	13	67
	10	+	49	16	28	55	234
	21	+	43	10	21	40	248
	52	+	53	27	26	65	239
	105	+	49	13	20	64	266
	210	+	28	14	26	71	269
	524	+	6	18	25	23	191

Table 7

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BENZO(a)PYRENE

EXPERIMENT 13

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	22	11	Contam.	57	148	73
		+	14	24	Contam.	54	180	69
Pre-Ozonation Benzo (a) Pyrene (saturated solution)	.25 ml	-	13	14	Contam.	75	172	40
	.25 ml	+	16	25	Contam.	70	184	43
Ozonation Benzo (a) Pyrene (saturated solution)	.005 ml	-	17	13	Contam.	56	154	68
	.01 ml	-	31	16	Contam.	51	178	59
	.025 ml	-	20	9	Contam.	59	154	42
	.05 ml	-	19	11	Contam.	46	164	58
	.10 ml	-	30	10	Contam.	48	176	54
	.25 ml	-	14	11	Contam.	55	146	64
	.005 ml	+	23	16	Contam.	63	224	54
	.01 ml	+	21	17	Contam.	50	186	63
	.025 ml	+	22	18	Contam.	58	214	68
	.05 ml	+	25	21	Contam.	64	175	54
	.10 ml	+	19	23	Contam.	45	204	62
	.25 ml	+	18	18	Contam.	63	160	42

Table 8

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BENZO(a)PYRENE

EXPERIMENT 27

Compound	Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	8	8	13	16	115	38
		+	10	10	14	45	131	52
Positive Control								
B-propiolactone	50 ug	-	328					
9 AA	100 ug	-		510				
2 NF	50 ug	-			618			
AF_2	0.1 ug	-				368	900	500
Pre-Ozonation	.25 ml	-	15	13	16	55	126	63
Benzo(a)Pyrene	.25 ml	+	13	12	22	42	144	74
Ozonation*	.005 ml	-	14	11	10	30	104	66
Benzo(a)Pyrene	.01 ml	-	23	7	8	52	107	81
	.025 ml	-	17	7	11	60	110	56
	.05 ml	-	5	15	12	39	124	67
	.1 ml	-	16	6	22	34	110	71
	.25 ml	-	17	11	11	48	137	65
	.005 ml	+	24	9	33	44	153	74
	.01 ml	+	15	13	24	44	148	72
	.025 ml	+	13	13	29	56	137	69
	.05 ml	+	11	16	27	50	121	57
	.1 ml	+	12	14	21	51	151	86
	.25 ml	+	12	8	26	52	142	69

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*100% of starting material reacted.

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

HMX

EXPERIMENT 27

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	8	8	13	16	115	38
		+	10	10	14	45	131	52
Positive								
B-propiolactone	50	-	328					
9 AA	100	-		510				
2 NF	50	-			618			
AF_2	0.1	-				368	900	500
Pre-Ozonation HMX	.08	-	21	8	19	46	134	42
	.08	+	16	10	22	41	131	72
Ozonation HMX	.001	-	33	6	11	33	111	45
	.002	-	20	7	9	25	132	51
	.005	-	27	6	13	39	136	45
	.01	-	24	8	11	35	120	59
	.02	-	20	6	19	41	112	38
	.04	-	20	7	12	52	131	37
	.001	+	6	17	28	44	101	71
	.002	+	16	12	22	54	124	58
	.005	+	14	6	21	51	111	--
	.01	+	22	8	22	37	145	74
	.02	+	12	13	12	38	101	52
	.04	+	20	11	17	42	119	--

Table 10

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

HMX

EXPERIMENT 30

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	41	36	27	25	116
		*+	21	48	32	38	123
Positive							
B-propiolactone	50	-	1200				
9 AA	100	-		210			
2 NF	50	-			54		
AF_2	0.1	-				248	500
2 AN	10	*+	120	~1300	~3000	1500	2200
Pre-Ozonation HMX	.07	-	40	40	14	23	111
	.07	*+	13	41	32	44	101
Ozonation HMX	.001	-	52	31	16	42	86
	.002	-	64	38	16	33	103
	.005	-	76	31	21	26	122
	.01	-	74	35	12	32	106
	.02	-	84	37	11	24	102
	.04	-	37	31	18	21	102
	.001	*+	12	40	17	32	111
	.002	+	21	43	16	40	109
	.005	+	16	41	25	35	83
	.01	+	18	48	18	38	112
	.02	+	18	42	31	36	89
	.04	+	17	41	24	39	104

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*All + MA plates contaminated.

Table 11

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
AND ESCHERICHIA COLI

RDX

EXPERIMENT 26

Compound	Amount of Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	18	5	7	36	80	80
		+	12	5	17	29	85	64
Positive Control								
B-propiolactone	50 ug	-	22					
9 AA	100 ug	-		161				
2 NF	50 ug	-			234			
AF_2	0.1 ug	-				Toxic	0	~1100
Pre-Ozonation RDX	.25 ml	-	7	0	7	39	95	77
	.25 ml	+	9	Toxic	11	31	97	67
Ozonation RDX (saturated solution)	.005 ml	-	10	3	12	21	65	59
	.01 ml	-	14	3	14	31	71	73
	.025 ml	-	14	2	12	20	97	54
	.05 ml	-	7	Toxic	6	28	83	67
	.01 ml	-	14	Toxic	8	31	77	53
	.25 ml	-	15	Toxic	6	22	91	66
	.005 ml	+	5	11	9	30	94	76
	.01 ml	+	7	4	8	30	86	102
	.025 ml	+	5	4	10	34	75	91
	.05 ml	+	1	Toxic	13	29	93	82
	.1 ml	+	9	Toxic	11	33	77	96
	.25 ml	+	4	Toxic	13	19	96	73

Table 12

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

RDX

EXPERIMENT 30

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	41	36	27	25	116
		+	21	48	32	38	123
Positive							
B-propiolactone	50	-	1200				
9 AA	100			210			
2 NF	50				54		
AF_2	0.1					248	500
2 AN	10	+	120	~1300	~3000	1500	2200
Pre-Ozonation*	7.9	-	37	33	19	31	112
RDX	7.9	+	16	55	37	23	91
Ozonation	0.2	-	42	39	14	29	99
RDX	0.3	-	20	47	20	30	110
	0.7	-	26	48	17	26	119
	1.5	-	31	47	16	30	119
	2.9	-	35	40	19	27	106
	7.4	-	39	30	22	26	88
	0.2	+	18	39	18	44	97
	0.3	+	9	48	27	35	101
	0.7	+	14	48	28	31	106
	1.5	+	6	57	23	42	93
	2.9	+	11	49	22	37	104
	7.4	+	12	47	31	38	92

Table 13

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

OLEIC ACID

EXPERIMENT 14

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	14	6	13	36	97	64
		+	27	16	15	57	109	57
Pre-Ozonation Oleic Acid (saturated solution)	.25 ml	-	23	6	3	31	103	50
	.25 ml	+	19	15	15	37	102	32
Ozonation Oleic Acid (saturated solution)	.005 ml	-	22	9	9	68	147	58
	.01 ml	-	14	8	12	63	150	50
	.025 ml	-	25	10	10	57	120	47
	.05 ml	-	20	8	10	64	128	56
	.10 ml	-	31	11	13	54	145	43
	.25 ml	-	18	3	6	10	112	47
	.005 ml	+	35	12	19	62	143	35
	.01 ml	+	30	15	21	57	122	56
	.025 ml	+	23	23	23	70	124	62
	.05 ml	+	21	22	23	56	148	49
	.10 ml	+	30	17	12	44	157	55
	.25 ml	+	16	21	14	20	113	59

Table 14

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

OLEIC ACID

EXPERIMENT 25

Compound	Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	23	17	9	39	143	64
		+	14	9	21	42	161	98
B-propiolactone	50 ug	-	~2500					
9 AA	100 ug	-		39				
2 NF	50 ug	-			~1200			
AF_2	0.1 ug	-				~700	0	~2000
Pre-Ozonation Oleic Acid	.25 ml	-	33	8	12	34	226	68
	.25 ml	+	12	12	17	58	142	85
Ozonation* Oleic Acid	.005 ml	-	33	19	7	32	214	59
	.01 ml	-	20	13	15	24	262	101
	.025 ml	-	49	10	8	46	205	86
	.05 ml	-	27	11	9	47	266	81
	.1 ml	-	28	20	18	36	235	98
	.25 ml	-	32	17	18	39	239	62
	.005 ml	+	20	11	32	66	158	131
	.01 ml	+	14	7	22	52	131	91
	.025 ml	+	11	22	25	57	160	98
	.05 ml	+	13	13	19	45	162	111
	.1 ml	+	21	20	27	41	168	84
	.25 ml	+	19	20	14	41	146	88

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*100% of starting material reacted.

Table 15

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

HUMIC ACIDS

EXPERIMENT 14

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					*E. coli*
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	14	6	13	36	97	64
		+	27	16	15	57	109	57
Pre-Ozonation Humic Acids (saturated solution)	.25 ml	-	31	13	8	54	118	67
	.25 ml	+	24	14	13	59	133	65
Ozonation Humic Acids (saturated solution)	.005 ml	-	70	5	19	46	136	42
	.01 ml	-	56	10	15	51	115	41
	.025 ml	-	33	8	16	74	114	65
	.05 ml	-	32	18	21	57	125	54
	.10 ml	-	41	10	21	53	123	68
	.25 ml	-	14	8	16	53	144	54
	.005 ml	+	27	20	15	58	143	68
	.01 ml	+	21	7	23	47	145	61
	.025 ml	+	19	15	13	67	144	67
	.05 ml	+	25	17	11	48	155	63
	.10 ml	+	19	16	21	56	145	87
	.25 ml	+	40	10	18	57	128	57

Table 16

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
AND ESCHERICHIA COLI

HUMIC ACIDS

EXPERIMENT 25

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100	E. Coli WP2
Negative Control		-	23	17	9	39	143	64
		+	14	9	21	42	161	98
Positive Control								
B-propiolactone	50 ug	-	~2500					
9 AA	100 ug	-		39				
2 NF	50 ug	-			~1200			
AF_2	0.1 ug	-				~700	0	~2000
Pre-Ozonation Humic Acids (saturated solution)	.25 ml	-	52	9	20	34	228	112
	.25 ml	+	14	16	23	62	208	102
Ozonation Humic Acids (saturated solution)	.005 ml	-	36	12	19	29	169	61
	.01 ml	-	32	13	15	32	164	75
	.025 ml	-	40	12	14	41	225	74
	.05 ml	-	35	14	15	48	223	74
	.1 ml	-	37	8	24	0	203	91
	.25 ml	-	46	11	10	42	265	99
	.005 ml	+	18	14	32	42	166	90
	.01 ml	+	15	15	29	35	148	130
	.025 ml	+	13	17	26	49	172	102
	.05 ml	+	6	8	25	68	128	89
	.1 ml	+	13	12	23	77	161	120
	.25 ml	+	13	14	22	42	158	73

Table 17

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

HUMIC ACIDS

EXPERIMENT 28

Compound	Compound Added Per Plate	MA	HISTIDINE REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA100
Negative Control		-	42	22	45	185
		+	32	22	60	246
Positive Control						
B-propiolactone	50 ug	-	1830			
2 AN	20 ug	+	2010	1515	6000	7000
9 AA	100 ug	-		1500		
AF_2	0.1 ug	-				~3000
2 NF	50 ug	-			1065	
Pre-Ozonation Humic Acids (saturated solution)	.25 ml	-	47	20	16	218
	.25 ml	+	27	24	26	220
Ozonation Humic Acids (saturated solution)	.005 ml	-	84	15	20	500
	.01 ml	-	64	17	17	216
	.025 ml	-	77	21	27	244
	.05 ml	-	78	11	23	300
	.1 ml	-	Contam.	10	29	254
	.25 ml	-	135	13	17	288
	.005 ml	+	39	19	37	488
	.01 ml	+	39	13	31	240
	.025 ml	+	41	16	36	250
	.05 ml	+	75	11	31	296
	.1 ml	+	83	16	27	312
	.25 ml	+	114	12	27	312

Table 18

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

HUMIC ACIDS

EXPERIMENT 33

Compound	Compound Added Per Plate	MA	HISTIDINE REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	20	15	9	85	117
		+	21	10	22	74	121
Positive Control							
B-propiolactone	50 ug	-	2400				
9 AA	100 ug	-		1000			
2 NF	50 ug	-			1500		
AF_2	0.1 ug	-				260	500
2 AN	20 ug	+	336	81	270	1200	2000
Pre-Ozonation Humic Acids (saturated solution)	.25 ml	-	14	9	14	55	130
	.25 ml	+	12	16	24	63	151
Ozonation Humic Acids (saturated solution)	.005 ml	-	16	11	10	68	135
	.01 ml	-	27	12	13	63	135
	.025 ml	-	15	16	12	67	150
	.05 ml	-	19	9	13	53	172
	.1 ml	-	18	11	18	55	133
	.25 ml	-	23	12	Contam.	62	117
	.005 ml	+	17	15	14	82	208
	.01 ml	+	22	13	21	77	157
	.025 ml	+	23	17	25	72	170
	.05 ml	+	21	21	24	73	186
	.1 ml	+	18	30	21	52	156
	.25 ml	+	10	9	19	70	170

Table 19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

TNT CONDENSATE WATER

EXPERIMENT 11

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	10	13	12	5	Contam.	19
		+	10	20	13	19	Contam.	48
Pre-Ozonation TNT Condensate Water	14.3	-	Contam.	Contam.	Contam.	Contam.	Contam.	Contam.
	14.3	+	Contam.	Contam.	Contam.	Contam.	Contam.	Contam.
Ozonation TNT Condensate Water	0.2	-	11	18	14	11	Contam.	34
	0.4	-	8	13	18	15	Contam.	27
	1.0	-	18	16	21	10	Contam.	35
	1.9	-	22	30	7	16	Contam.	46
	3.9	-	9	13	24	14	Contam.	41
	9.7	-	9	21	10	17	Contam.	31
	0.2	+	19	22	25	18	Contam.	37
	0.4	+	13	22	27	16	Contam.	33
	1.0	+	11	14	30	19	Contam.	30
	1.9	+	18	29	23	28	Contam.	36
	3.9	+	13	23	36	23	Contam.	57
	9.7	+	20	29	17	25	Contam.	46

Table 20

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

TNT CONDENSATE WATER

EXPERIMENT 24

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	16	10	8	30	173	85
		+	13	14	22	41	190	79
Positive Control								
AF$_2$	0.1	-				48	~200	82
B-propiolactone	50	-	144					
9 AA	100	-		136				
2 NF	50	-			500			
Pre-Ozonation	8.9	-	800	24	1000	700	1280	12
TNT Condensate Water	8.9	+	800	~750	500	50	500	90
Ozonation	0.2	-	17	13	12	42	180	78
TNT Condensate Water	0.3	-	13	10	12	26	184	74
	0.8	-	11	13	12	39	156	90
	1.6	-	28	11	13	33	202	79
	3.2	-	15	12	13	29	206	70
	8.0	-	22	13	23	47	234	86
	0.2	+	50	14	18	55	148	77
	0.3	+	15	10	18	53	194	86
	0.8	+	20	23	16	50	186	96
	1.6	+	12	17	21	65	196	66
	3.2	+	17	12	16	58	189	62
	8.0	+	14	14	15	47	160	75

Table 21

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DIPHENYLHYDRAZINE

EXPERIMENT 26

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	18	5	7	36	80	80
		+	12	5	17	29	85 c	64
B-propiolactone	50	-	22					
9 AA	100	-		161				
2 NF	50	-			234			
AF_2	0.1	-				pp	0	~1100
Pre-Ozonation	2048	-	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
Diphenylhydrazine	2048	+	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
Ozonation	22	-	14	6	15	21	64	77
Diphenylhydrazine	54	-	10	2	12	27	87	71
	135	-	15	10	3	28	89	95
	270	-	18	2	12	22	88	70
	541	-	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	1351	-	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
	22	+	12	10	27	34	106	97
	54	+	9	7	22	25 c	91	91
	135	+	12	11	17	24 c	75	94
	270	+	14	10	23	26 c	91	87
	541	+	8	3	11	19 c	95	88
	1351	+	8	Toxic	Toxic	Toxic	Toxic	Toxic

Table 22

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

DIPHENYLHYDRAZINE

EXPERIMENT 29

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	25	23	5	35	82
		+	12	12	23	44	122
Positive Control							
B-propiolactone	50	-	~950				
9 AA	100	-		450			
2 NF	50	-			~400		
AF_2	0.1	-				~600	~1800
2 AN	10	+	83	~600	~3000	~1500	~5000
Pre-Ozonation Diphenylhydrazine	23	-	49	14	12	35	136
	45	-	35	15	14	34	125
	113	-	37	17	20	27	118
	226	-	27	16	18	26	104
	452	-	30	13	24	18	106
	1131	-	Toxic	Toxic	Toxic	Toxic	Toxic
	23	+	9	19	31	43	148
	45	+	18	21	14	34	154
	113	+	16	18	19	Contam.	121
	226	+	10	20	29	44	143
	452	+	27	7	25	37	97
	1131	+	20	30	19	34	83
Ozonation Diphenylhydrazine	5	-	28	11	19	24	87
	10	-	30	12	18	26	136
	25	-	26	16	19	35	127
	50	-	32	14	18	38	135
	100	-	37	18	19	31	104
	249	-	Toxic	Toxic	Toxic	Toxic	Toxic
	5	+	11	18	31	27	129
	10	+	11	17	29	31	116
	25	+	19	18	25	36	118
	50	+	14	21	23	39	102
	100	+	17	10	21	32	145
	249	+	18	10	23	34	107

Table 23

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

DIPHENYLHYDRAZINE

EXPERIMENT 34

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	17	11	14	14	164
		+	8	9	15	20	147
Positive Control							
B-propiolactone	50	-	650				
9 AA	100	-		250			
2 NF	50	-			291		
AF_2	0.1	-				21	560
2 AN	10	+	10	430	1600	94	1500
Pre-Ozonation Diphenylhydrazine	112	-	30	6	5	10	156
	112	+	9	10	20	18	160
Ozonation Diphenylhydrazine	0.4	-	19	7	19	9	170
	0.8	-	38	9	7	13	165
	2	-	30	6	10	3	200
	4	-	35	9	10	14	154
	8	-	38	10	9	10	200
	20	-	67	9	16	8	207
	0.4	+	4	9	12	14	181
	0.8	+	7	7	16	20	177
	2	+	14	10	16	10	183
	4	+	12	8	12	16	202
	8	+	8	7	14	24	211
	20	+	10	7	12	14	212

Table 24

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

NITRILOTRIACETIC ACID, TRISODIUM SALT MONOHYDRATE

EXPERIMENT 13

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control								
		-	22	11	Contam.	57	148	73
		+	14	24	Contam.	54	180	69
Pre-Ozonation NTA	3369	-	22	8	Contam.	53	182	23
		+	21	15	Contam.	54	142	42
Ozonation NTA	34	-	23	13	Contam.	45	190	29
	69	-	22	22	Contam.	53	174	28
	172	-	19	15	Contam.	50	126	16
	344	-	26	21	Contam.	52	144	32
	687	-	20	18	Contam.	44	200	24
	1718	-	16	18	Contam.	56	144	26
	34	+	23	13	Contam.	55	160	21
	69	+	18	25	Contam.	72	204	51
	172	+	16	19	Contam.	52	172	77
	344	+	17	19	Contam.	66	163	82
	687	+	17	25	Contam.	51	190	58
	1718	+	19	28	Contam.	65	184	74

Table 25

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

NITRILOTRIACETIC ACID, TRISODIUM SALT MONOHYDRATE

EXPERIMENT 23

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	31	12	16	43	210	85
		+	Contam.	15	Contam.	Contam.	175	138
B-propiolactone	50	-	~420					
9 AA	100	-		~500				
2 NF	50	-			~600			
AF_2	0.1	-				62	360	139
Pre-Ozonation Nitrilioacetic Acid	2613	-	42	12	19	42	158	69
	2613	+	13	11	28	36	149	63
Ozonation Nitrilioacetic Acid	26	-	22	11	6	35	176	107
	52	-	39	10	8	35	172	96
	131	-	31	18	16	50	188	106
	261	-	28	10	11	34	148	102
	523	-	35	11	8	48	201	117
	1306	-	43	11	18	54	167	81
	26	+	35	13	24	41	167	88
	52	+	24	20	Contam.	49	168	108
	131	+	15	10	15	48	181	106
	261	+	Contam.	8	9	50	177	82
	523	+	25	12	Contam.	55	177	88
	1306	+	Contam.	15	22	56	200	99

Table 26

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND ESCHERICHIA COLI

ALDRIN

EXPERIMENT 12

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	20	20	11	21	Contam.	33
		+	33	15	32	30	Contam.	48
Pre-Ozonation Aldrin (saturated solution)	.25 ml	-	Contam.	Contam.	Contam.	Contam.	Contam.	Contam.
	.25 ml	+	Contam.	Contam.	Contam.	Contam.	Contam.	Contam.
Ozonation Aldrin (saturated solution)	.005 ml	-	16	13	10	15	Contam.	18
	.01 ml	-	16	12	11	18	Contam.	12
	.025 ml	-	12	16	11	23	Contam.	24
	.05 ml	-	12	15	14	14	Contam.	32
	.10 ml	-	16	12	11	18	Contam.	35
	.25 ml	-	15	10	18	18	Contam.	31
	.005 ml	+	9	16	22	21	Contam.	29
	.01 ml	+	13	11	22	19	Contam.	25
	.025 ml	+	16	20	24	32	Contam.	29
	.05 ml	+	11	21	26	20	Contam.	30
	.10 ml	+	16	13	51	20	Contam.	43
	.25 ml	+	13	20	37	30	Contam.	38

Table 27

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ALDRIN

EXPERIMENT 23

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	31	12	16	43	210	85
		+	Contam.	15	Contam.	Contam.	175	138
Positive Control								
B-propiolactone	50 ug	-	~420					
9 AA	100 ug	-		~500				
2 NF	50 ug	-			~600			
AF_2	0.1 ug	-				62	360	139
Pre-Ozonation Aldrin (saturated solution)	.25 ml	-	34	7	6	32	156	64
	.25 ml	+	13	9	14	41	167	100
Ozonation Aldrin (saturated solution)	.005 ml	-	22	12	19	29	159	71
	.01 ml	-	27	4	11	29	150	46
	.025 ml	-	22	12	19	46	184	67
	.05 ml	-	32	6	17	40	172	75
	.1 ml	-	19	7	11	42	176	61
	.25 ml	-	49	13	6	43	156	56
	.005 ml	+	Contam.	Contam.	29	51	143	106
	.01 ml	+	16	15	26	57	138	132
	.025 ml	+	12	6	35	38	153	113
	.05 ml	+	10	17	19	38	123	90
	.1 ml	+	16	10	13	36	146	90
	.25 ml	+	16	13	20	36	146	83

Table 28

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DDT

EXPERIMENT 12

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	20	20	11	21	Contam.	33
		+	33	15	32	30	Contam.	48
Pre-Ozonation DDT (saturated solution)	.25 ml	-	126	135	158	174	Contam.	168
	.25 ml	+	100	136	140	150	Contam.	233
Ozonation DDT (saturated solution)	.005 ml	-	23	12	13	32	Contam.	48
	.01 ml	-	25	18	6	20	Contam.	53
	.025 ml	-	26	17	10	19	Contam.	30
	.05 ml	-	23	21	15	26	Contam.	36
	.10 ml	-	28	18	11	24	Contam.	31
	.25 ml	-	23	13	15	17	Contam.	42
	.005 ml	+	22	28	40	25	Contam.	57
	.01 ml	+	25	25	45	20	Contam.	45
	.025 ml	+	23	23	40	23	Contam.	46
	.05 ml	+	22	27	32	23	Contam.	52
	.10 ml	+	20	17	25	24	Contam.	77
	.25 ml	+	21	33	50	22	Contam.	53

Table 29

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

DDT

EXPERIMENT 23

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	31	12	16	43	210	85
		+	Contam.	15	Contam.	Contam.	175	138
Positive Control								
B-propiolactone	50 ug	-	~420					
9 AA	100 ug	-		~500				
2 NF	50 ug	-			~600			
AF_2	0.1 ug	-				62	360	139
Pre-Ozonation DDT (saturated solution)	.25 ml	-	29	10	18	39	169	84
	.25 ml	+	16	7	Contam.	50	153	75
Ozonation DDT (saturated solution)	.005 ml	-	35	6	8	29	164	106
	.01 ml	-	32	12	11	30	146	74
	.025 ml	-	40	7	17	38	162	102
	.05 ml	-	43	11	10	28	151	77
	.1 ml	-	29	10	21	46	145	101
	.25 ml	-	36	7	16	34	155	89
	.005 ml	+	Contam.	11	Contam.	50	164	112
	.01 ml	+	Contam.	15	27	49	183	109
	.025 ml	+	26	10	20	54	181	79
	.05 ml	+	27	7	19	43	162	88
	.1 ml	+	17	11	Contam.	45	186	75
	.25 ml	+	Contam.	10	8	52	155	83

Table 30

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

AROCHLOR 1254

EXPERIMENT 10

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. col
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	9	4	13	15	106	37
		+	9	22	26	6	100	31
Pre-Ozonation Arochlor 1254 (saturated solution)	.25 ml	-	15	10	16	8	92	16
	.25 ml	+	14	17	13	16	86	34
Ozonation Arochlor 1254 (saturated solution)	.005 ml	-	11	14	15	9	113	23
	.01 ml	-	6	10	21	11	109	35
	.025 ml	-	17	10	16	17	111	42
	.050 ml	-	9	13	9	14	102	49
	.10 ml	-	15	10	18	12	101	47
	.25 ml	-	9	15	15	11	99	120
	.005 ml	+	16	21	26	22	121	41
	.01 ml	+	10	20	28	18	115	32
	.025 ml	+	9	15	15	31	106	47
	.050 ml	+	18	21	21	17	92	76
	.10 ml	+	14	6	27	16	100	93
	.25 ml	+	13	22	23	22	97	197

Table 31

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

AROCHLOR 1254

EXPERIMENT 23

Compound	Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	32	12	16	43	210	85
		+	Contam.	15	Contam.	Contam.	175	138
B-propiolactone	50 ug	-	420					
9 AA	100 ug	-		500				
2 NF	50 ug	-			600			
AF_2	0.1 ug	-				62	360	139
Pre-Ozonation Arochlor (saturated solution)	.25 ml	-	49	14	25	27	165	124
	.25 ml	+	32	16	Contam.	58	164	115
Ozonation Arochlor (saturated solution)	.005 ml	-	39	6	13	27	197	108
	.01 ml	-	42	4	18	38	151	91
	.025 ml	-	36	15	9	37	182	90
	.05 ml	-	33	8	15	44	157	94
	.1 ml	-	31	14	24	36	188	99
	.25 ml	-	35	8	16	31	186	114
	.005 ml	+	14	10	Contam.	54	163	109
	.01 ml	+	15	20	Contam.	39	137	108
	.025 ml	+	Contam.	11	33	47	156	121
	.05 ml	+	Contam.	10	Contam.	54	144	130
	.1 ml	+	17	16	Contam.	57	134	135
	.25 ml	+	19	12	31	67	158	122

Table 32

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

ACETIC ACID

EXPERIMENT 2

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	36	14	18	21	185
		+	14	16	37	24	197
Pre-Ozonation Acetic Acid	2500	-	66	12	7	20	144
	2500	+	14	8	18	21	154
Ozonation Acetic Acid	2500	-	23	8	9	11	348
	2500	+	10	25	17	62	368

Table 33

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

ACETIC ACID

EXPERIMENT 23

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. Typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	8	3	6	25	184	71
		+	10	8	21	31	148	78
Positive								
B-propiolactone	50	-	352					
9 AA	100	-		1000				
2 NF	50	-			426			
AF_2	0.1	-				132	1000	754
Pre-Ozonation Acetic Acid	3150	-	18	9	12	27	106	52
	3150	+	4	8	12	34	100	58
Ozonation Acetic Acid	61	-	16	7	12	42	146	100
	122	-	11	7	9	28	141	Contam.
	304	-	17	7	7	41	140	57
	608	-	11	6	7	29	112	98
	1216	-	20	4	10	28	115	66
	3040	-	10	13	11	21	134	67
	61	+	6	13	12	23	150	93
	122	+	10	12	11	22	174	78
	304	+	3	6	19	13	140	77
	608	+	10	12	7	35	105	82
	1216	+	7	15	18	34	128	66
	3040	+	8	11	13	28	112	52

Table 34

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

GLUCOSE

EXPERIMENT 10

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	9	4	13	15	106	37
		+	9	22	26	6	100	31
Pre-Ozonation Glucose	2500 ug	-	14	5	14	11	90	21
	2500 ug	+	13	13	17	19	118	26
Ozonation* Glucose	.005 ml	-	15	11	22	12	103	26
	.01 ml	-	12	12	16	10	127	39
	.025 ml	-	4	13	10	9	102	29
	.05 ml	-	12	7	13	10		41
	.10 ml	-	10	14	14	15	117	24
	.25 ml	-	9	10	18	23	138	24
	.005 ml	+	10	13	25	17	118	37
	.01 ml	+	11	15	26	20	100	38
	.025 ml	+	15	10	21	17	98	32
	.05 ml	+	18	19	22	15	102	39
	.10 ml	+	8	14	16	9	113	21
	.25 ml	+	20	17	16	18	133	22

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*100% of starting material reacted.

Table 35

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

GLUCOSE

EXPERIMENT 21

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control								
		-	28	13	16	25	105	84
		+	20	15	21	40	131	84
Positive Control								
AF_2	0.1 ug					290	900	1200
B-propiolactone	50 ug		450					
9 AA	100 ug			680				
2 NF	50 ug				354			
Pre-Ozonation	.25 ml	-	18	25	27	23	127	83
Glucose	.25 ml	+	25	18	15	23	94	87
Ozonation*	.005 ml	-	23	12	17	20	81	89
Glucose	.01 ml	-	21	10	18	18	109	58
	.025 ml	-	19	10	18	28	100	74
	.05 ml	-	18	9	15	23	Toxic	58
	.1 ml	-	20	12	11	16	Toxic	78
	.25 ml	-	15	18	20	8	Toxic	65
	.005 ml	+	18	19	16	30	152	97
	.01 ml	+	8	8	28	25	154	73
	.025 ml	+	6	15	13	Toxic	113	76
	.05 ml	+	12	18	19	25	Toxic	84
	.1 ml	+	14	16	10	25	Toxic	72
	.25 ml	+	9	Contam.	17	14	Toxic	73

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*100% of starting material reacted.

Table 36

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

UREA

EXPERIMENT 9

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					*E. coli*
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		–	22	17	9	23	73	55
		+	30	41	18	33	75	40
Pre-Ozonation Urea	2500	–	12	11	11	13	28	27
	2500	+	20	29	12	36	95	54
Ozonation Urea	50	–	32	18	18	18	50	42
	100	–	22	18	9	13	79	26
	250	–	22	21	14	17	58	59
	500	–	29	9	16	18	54	64
	1000	–	21	23	15	17	39	57
	2500	–	27	12	14	11	76	47
	50	+	26	50	20	24	125	47
	100	+	30	40	48	19	111	58
	250	+	29	36	17	35	78	61
	500	+	32	47	26	30	93	65
	1000	+	19	43	29	16	99	70
	2500	+	27	26	33	31	93	69

Table 37

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

UREA

EXPERIMENT 21

Compound	Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		–	28	13	16	25	105	84
		+	20	15	21	40	131	84
Positive Control								
AF_2	0.1	–				290	900	1200
B-propiolactone	50	–	450					
9 AA	100	–		680				
2 NF	50	–			354			
Pre-Ozonation Urea	2350	–	12	17	24	15	117	63
	2350	+	15	12	24	18	111	100
Ozonation Urea	45	–	11	10	10	20	108	74
	90	–	21	8	14	9	128	57
	226	–	21	17	12	16	124	70
	452	–	15	9	7	8	108	72
	904	–	22	14	Toxic	13	92	65
	2261	–	16	10	Toxic	21	130	75
	45	+	14	18	33	29	77	105
	90	+	11	18	20	25	128	67
	226	+	8	11	14	14	142	78
	452	+	12	13	21	23	90	68
	904	+	13	14	19	Toxic	116	61
	2261	+	11	14	11	Toxic	109	79

Table 38

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

CHOLESTEROL

EXPERIMENT 8

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				E. coli
			TA1535	TA1537	TA1538	TA98	WP2
Negative Control		-	14	8	22	20	41
		+	12	28	30	40	56
Pre-Ozonation Cholesterol (saturated solution)	.25 ml	-	160	157	147	131	198
	.25 ml	+	152	174	140	120	178
Ozonation Cholesterol (saturated solution)	.005 ml	-	8	11	14	10	52
	.01 ml	-	22	14	20	11	44
	.025 ml	-	19	21	27	16	50
	.05 ml	-	18	12	13	14	42
	.10 ml	-	14	10	12	15	54
	.25 ml	-	16	9	7	15	46
	.005 ml	+	13	23	19	40	60
	.01 ml	+	18	12	23	29	74
	.025 ml	+	13	15	16	34	53
	.05 ml	+	14	19	24	26	60
	.10 ml	+	19	23	22	16	67
	.25 ml	+	15	22	20	27	54

Table 39

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CHOLESTEROL

EXPERIMENT 20

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	21	3	7	31	114	64
		+	17	9	16	30	133	37
Positive Control								
B-propiolactone	50 ug	-	358					
9 AA	100 ug	-		869				
2 NF	50 ug	-			307			
AF_2	0.1 ug	-				184	1000	471
Pre-Ozonation Cholesterol (saturated solution)	.25 ml	-	33	11	20	37	180	35
	.25 ml	+	17	11	22	39	157	122
Ozonation Cholesterol (saturated solution)	.005 ml	-	38	9	11	31	162	103
	.01 ml	-	45	7	15	27	178	101
	.025 ml	-	32	14	12	35	186	97
	.05 ml	-	42	11	18	35	164	88
	.1 ml	-	43	9	19	32	175	32
	.25 ml	-	41	8	12	35	154	38
	.05 ml	+	11	9	24	49	134	128
	.01 ml	+	11	14	27	48	150	99
	.025 ml	+	13	12	22	39	121	101
	.05 ml	+	17	9	13	38	142	152
	.1 ml	+	21	10	26	49	124	120
	.25 ml	+	13	10	16	21	146	125

Table 40

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

BENZIDINE

EXPERIMENT 9

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	22	17	9	23	73	55
		+	30	41	18	33	75	40
Pre-Ozonation Benzidine	1250	-	24	17	10		88	30
	1250	+	toxic	350	145	460	128	39
Ozonation Benzidine	11	-	17	25	18	14	30	54
	21	-	12	31	17	20	28	48
	51	-	25	55	16	42	36	43
	103	-	25	64	21	53	46	54
	205	-	13	95	29	78	101	53
	513	-	8	97	24	98	152	56
	11	+	34	133	108	171	45	71
	21	+	43	168	123	100	22	65
	51	+	30	227	160	270	81	54
	103	+	22	120	120	320	121	60
	205	+	21	214	125	260	92	48
	513	+	7	92	38	208	123	38

Table 41

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BENZIDINE

EXPERIMENT 20

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	21	3	7	31	114	64
		+	17	9	16	30	133	37
Positive								
B-propiolactone	50	-	358					
9 AA	100	-		869				
2 NF	50	-			307			
AF_2	0.1	-				184	>1000	471
Pre-Ozonation Benzidine	1200	-	39	6	16	33	158	66
	1200	+	13	14	253	148	285	40
Ozonation Benzidine	9	-	44	8	28	26	180	83
	17	-	28	8	39	31	152	99
	43	-	30	12	44	45	167	91
	85	-	29	13	72	45	219	95
	171	-	27	17	119	75	241	87
	427	-	30	9	148	68	298	94
	9	+	18	15	26	61	171	113
	17	+	16	16	53	64	186	115
	43	+	14	12	97	125	192	104
	85	+	19	26	133	157	224	116
	171	+	20	36	142	115	190	126
	427	+	18	12	139	71	201	36

Table 42

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

BENZIDINE

EXPERIMENT 36

Compound	Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	27	12	15	25	110
		+	10	14	26	39	135
Positive Control							
B-propiolactone	50 ug	-	1030				
9 AA	100 ug	-		260			
2 NF	50 ug	-			500		
AF_2	0.1 ug	-				304	760
2 AN	20 ug	+	340	330	690	1580	1760
Pre-Ozonation Benzidine	.25 ml	-	25	12	17	36	102
	.25 ml	+	15	6	38	44	101
Ozonation* Benzidine	.005 ml	-	18	8	9	30	101
	.01 ml	-	50	13	20	16	92
	.025 ml	-	36	10	20	39	90
	.05 ml	-	49	8	20	61	86
	.1 ml	-	27	4	25	66	87
	.25 ml	-	28	14	52	131	113
	.005 ml	+	11	15	35	38	100
	.01 ml	+	20	16	29	42	116
	.025 ml	+	19	13	29	43	75
	.05 ml	+	19	20	31	45	96
	.1 ml	+	11	15	20	50	116
	.25 ml	+	17	14	29	65	87

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*100% of starting material reacted.

Table 43

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

BENZIDINE

EXPERIMENT 36

Compound	Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	27	12	15	25	110
		+	10	14	26	39	135
Positive Control							
B-propiolactone	50 ug	-	1030				
9 AA	100 ug	-		260			
2 NF	50 ug	-			500		
AF_2	0.1 ug	-				304	760
2 AN	20 ug	+	340	330	690	1580	1760
Pre-Ozonation Benzidine	.25 ml	-	23	7	13	29	140
	.25 ml	+	10	9	14	21	160
Ozonation* Benzidine	.005 ml	-	39	8	25	106	100
	.01 ml	-	29	5	44	200	128
	.025 ml	-	17	4	78	460	Contam.
	.05 ml	-	32	16	148	760	119
	.1 ml	-	21	12	235	1050	150
	.25 ml	-	29	16	Contam.	1550	Contam.
	.005 ml	+	16	11	22	59	92
	.01 ml	+	11	14	35	75	117
	.025 ml	+	14	8	33	163	122
	.05 ml	+	13	19	46	235	140
	.1 ml	+	6	10	72	409	121
	.25 ml	+	10	10	171	840	155

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*100% of starting material reacted.

Table 44

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

GLYCINE

EXPERIMENT 7

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				E. coli
			TA1535	TA1537	TA1538	TA98	WP2
Negative Control		-	14	16	15	15	71
		+	15	11	19	32	94
Pre-Ozonation Glycine	2500	-	160	157	147	131	56
	2500	+	152	174	120	120	28
Ozonation Glycine	40	-	24	Contam.	22	17	62
	79	-	21	14	14	12	54
	198	-	16	10	12	18	48
	395	-	16	12	11	14	55
	790	-	20	16	21	17	44
	1975	-	14	15	10	18	53
	40	+	11	17	26	17	62
	79	+	16	18	37	24	65
	198	+	16	30	38	33	83
	395	+	15	16	39	36	79
	790	+	9	11	35	28	80
	1975	+	11	10	44	30	58

Table 45

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

GLYCINE

EXPERIMENT 20

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	21	3	7	31	114	64
		+	17	9	16	30	133	37
Positive								
B-propiolactone	50	-	358					
9 AA	100	-		869				
2 NF	50	-			307			
AF_2	0.1	-				184	>1000	471
Pre-Ozonation	2975	-	109	108	106	110	276	182
Glycine	2975	+	28	108	43	116	208	184
Ozonation	46	-	42	7	14	32	194	87
Glycine	93	-	31	13	14	35	173	72
	232	-	40	10	9	34	206	87
	464	-	35	5	10	41	185	93
	928	-	25	15	10	27	172	62
	2321	-	26	17	13	35	186	94
	46	+	17	13	18	61	220	129
	93	+	10	11	31	39	132	126
	232	+	14	13	11	54	132	129
	464	+	10	15	15	44	128	116
	928	+	10	22	25	38	137	136
	2321	+	10	8	24	53	164	144

Table 46

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

GLYCINE

EXPERIMENT 30

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		–	41	36	27	25	116
		+	21	48	32	38	123
Positive							
B-propiolactone	50	–	1200				
9 AA	100	–		210			
2 NF	50	–			54		
AF_2	0.1	–				248	500
2 AN	20	+	120	~1300	~3000	1500	2200
Pre-Ozonation Glycine	2725	–	35	47	16	23	116
	2725	+	13	56	25	31	92
Ozonation Glycine	19	–	40	36	18	20	102
	37	–	35	32	15	21	94
	93	–	29	45	18	32	117
	185	–	26	51	21	30	105
	371	–	26	42	22	25	122
	927	–	21	46	22	32	102
	19	+	11	46	30	32	93
	37	+	20	43	19	35	92
	93	+	21	43	22	40	93
	185	+	7	52	23	43	121
	371	+	15	47	17	34	109
	927	+	19	52	17	49	89

Table 47

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

BENZENE

EXPERIMENT 4

Compound	Amount of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	25	16	7	15	134	33
		+	17	15	18	31	136	46
Pre-Ozonation Benzene	175	-	154	16	14	12	124	40
	175 µg	+	10	15	14	17	145	41
Ozonation* Benzene	.005 µl	-	44	16	17	7	124	27
	.01	-	70	12	16	11	143	23
	.025	-	49	10	11	12	153	30
	.05	-	43	12	14	18	124	4
	.1	-	53	10	17	10	139	17
	.25	-	87	14	18	10	173	16
	.005	+	12	28	21	22	152	46
	.01	+	14	18	19	20	172	9
	.025	+	10	10	19	26	124	26
	.05	+	16	18	21	19	141	24
	.1	+	8	27	19	16	132	Contam.
	.25	+	12	22	19	19	158	20

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* % Reacted not quantitated.

Table 48

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

BENZENE

EXPERIMENT 19

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	16	5	9	32	134	102
		+	20	5	28	47	138	118
Positive Control								
B-propiolactone	50	-	190					
4-o-Tolylazo-o-toluidine	25	+			163			
AF_2	.1					211	700	600
Pre-Ozonation Benzene	137	-	50	Contam.	Contam.	Contam.	Contam.	80
	137	+	13	Contam.	18	29	97	93
Ozonation Benzene	2	-	12	10	Contam.	25	104	90
	4	-	6	4	10	34	93	81
	10	-	14	5	4	Contam.	90	94
	20	-	18	3	12	24	114	97
	41	-	15	10	17	20	102	Contam.
	102	-	15	Toxic	15	27	Contam.	Contam.
	2	+	43	5	Contam.	0	95	86
	4	+	38	6	Contam.	Contam.	83	98
	10	+	47	Contam.	Contam.	35	Contam.	132
	20	+	33	5	Contam.	30	105	103
	41	+	40	3	Contam.	Contam.	Contam.	98
	102	+	38	Contam.	15	40	Contam.	111

Table 49

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

CYSTEINE

EXPERIMENT 7

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				E. coli
			TA1535	TA1537	TA1538	TA98	WP2
Negative Control		-	14	16	15	15	71
		+	15	11	19	32	94
Pre-Ozonation Cysteine	2500	-	6	9	9	9	40
	2500	+	12	10	15	26	57
Ozonation Cysteine	6	-	15	20	18	12	54
	11	-	15	17	18	12	49
	28	-	13	13	15	12	48
	55	-	15	14	18	17	64
	110	-	12	12	14	20	46
	275	-	4	5	9	15	34
	6	+	12	10	20	16	67
	11	+	12	7	18	27	62
	28	+	11	9	22	12	56
	55	+	14	11	26	26	60
	110	+	8	12	20	15	60
	275	+	3	8	7	9	42

Table 50

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CYSTEINE

EXPERIMENT 20

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	21	3	7	31	114	64
		+	17	9	16	30	133	37
Positive								
B-propiolactone	50	-	358					
9 AA	100	-		869				
2 NF	50	-			307			
AF_2	0.1	-				184	>1000	471
Pre-Ozonation Cysteine	2400	-	14	0	12	16	133	93
	2400	+	13	0	21	48	333	78
Ozonation Cysteine	40	-	14	0	17	37	154	87
	79	-	8	0	14	40	134	71
	198	-	11	0	13	30	162	77
	396	-	10	0	18	38	140	90
	791	-	3	0	11	65	143	58
	1976	-	1	0	13	29	143	91
	40	+	9	0	24	36	129	100
	79	+	16	0	32	38	178	92
	198	+	7	0	29	41	143	86
	396	+	4	0	16	48	200	60
	791	+	7	0	27	43	136	73
	1976	+	7	0	19	49	156	78

Table 51

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

THYMINE

EXPERIMENT 19

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	16	5	9	32	134	102
		+	20	5	28	47	138	118
Positive Control								
B-propiolactone	50	-	190					
4-o-Tolylazo-o-toluidine	100	+			163			
AF_2	.1	-				211	700	600
Pre-Ozonation Thymine	575	-	67	7	10	0	Contam.	Contam.
	575	+	23	9	Contam.	82	Contam.	Contam.
Ozonation Thymine	0.7	-	48	7	Contam.	43	94	77
	1.4	-	Contam.	7	Contam.	33	114	92
	3.5	-	51	9	Contam.	46	115	84
	6.9	-	34	8	Contam.	31	91	75
	13.8	-	28	3	Contam.	14	82	90
	34.5	-	2	1	Contam.	0	0	67
	0.7	+	13	11	Contam.	0	78	C
	1.4	+	14	5	Contam.	0	89	94
	3.5	+	17	3	Contam.	Contam.	74	103
	6.9	+	19	6	Contam.	44	88	Contam.
	13.8	+	10	7	Contam.	36	79	Contam.
	34.5	+	Contam.	3	Contam.	Toxic	60	Contam.

Table 52

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

THYMINE

EXPERIMENT 39

Compound	Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	13	3	13	23	99
		+	8	4	67	57	136
Positive Control							
B-Propiolactone		-	146				
9AA		-		630			
2NF		-			350		
AF2		-				337	974
2An		-	21	3	15	25	162
		+	34	32	233	310	490
Preozonation	575	-	18	2	6	6	35
Thymine	575	+	14	4	10	11	67
Ozonation	29	-	7	1	11	24	120
Thymine	59	-	13	10	11	13	101
	88	-	15	2	6	1	102
	118	-	5	0	3	0	75
	147	-	0	0	0	0	36
	29	+	7	5	11	24	100
	59	+	14	6	12	27	108
	88	+	9	1	17	17	19
	118	+	8	7	17	30	49
	147	+	14	5	26	20	80

Table 53

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CAFFEINE

EXPERIMENT 6

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE				
			S. typhimurium				E. coli
			TA1535	TA1537	TA1538	TA98	WP2
Negative Control		-	14	8	22	20	41
		+	12	28	30	40	56
Pre-Ozonation Caffeine	2500	-	18	19	19	19	56
	2500	+	13	19	29	11	28
Ozonation Caffeine	32	-	15	25	20	16	53
	63	-	23	14	15	24	62
	158	-	12	16	20	17	65
	315	-	14	15	29	14	63
	630	-	18	20	22	19	43
	1575	-	21	15	21	13	42
	32	+	25	18	27	31	56
	63	+	17	22	26	42	42
	158	+	16	13	23	27	52
	315	+	23	23	27	38	55
	630	+	11	24	25	27	44
	1575	+	12	24	22	23	51

Table 54

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

CAFFEINE

EXPERIMENT 18

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	63	9	16	30	312	80
		+	17	19	21	44	336	100
Positive								
2-nitrofluorene	50	-			~1000			
B-propiolactone	50	-	300					
AF_2	0.1	-				272	1000	600
Pre-Ozonation Caffeine	2425	-	~1500	Contam.	Contam.	Contam.	264	
	2425	+	~1500	Contam.	Contam.	Contam.	405	
Ozonation Caffeine	19	-	48	10	10	42	215	66
	38	-	41	8	13	39	204	77
	95	-	40	8	19	59	183	71
	189	-	34	8	12	44	197	60
	378	-	31	8	11	41	158	76
	946	-	47	12	17	49	170	75
	19	+	12	15	20	48	252	Contam.
	38	+	20	12	14	46	235	67
	95	+	21	15	13	50	241	67
	189	+	22	14	15	53	181	63
	378	+	18	6	18	38	149	69
	946	+	19	11	19	54	147	92

Table 55

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

DIETHYLAMINE

EXPERIMENT 18

Compound	Micrograms of Compound Added per Plate	MA	REVERTANTS PER PLATS					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	63	9	16	30	312	80
		+	17	19	21	44	336	100
Positive Control								
2-nitrofluorene	100	-			~1000			
B-propiolactone	50	-	300					
AF_2	0.1	-				272	1000	600
Pre-Ozonation Diethylamine	2000	-	36	1	Toxic	23	150	61
	2000	+	64	13	15	30	166	~74
Ozonation Diethylamine	25	-	46	6	14	40	188	77
	51	-	45	Toxic	10	36	185	60
	127	-	46	5	14	40	208	80
	254	-	40	6	17	34	212	70
	508	-	31	6	12	48	163	66
	1270	-	22	5	Toxic	Toxic	256	57
	25	+	25	13	20	430	280	87
	51	+	19	15	24	35	301	79
	127	+	12	10	21	54	189	97
	254	+	19	12	14	44	195	67
	508	+	36	11	25	44	179	66
	1270	+	34	5	16	48	187	99

Table 56

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

DIETHYLAMINE

EXPERIMENT 29

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	25	23	5	35	82
		+	12	12	23	44	122
Positive							
B-propiolactone	50	-	~950				
9 AA	100	-		450			
2 NF	50	-			~400		
AF_2	0.1	-				~600	~1800
2 AN	20	+	83	~600	~3000	~1500	~5000
Pre-Ozonation Diethylamine	35	-	33	8	15	30	114
	71	-	30	16	16	22	108
	177	-	26	7	10	24	124
	353	-	25	9	7	24	86
	706	-	20	5	12	25	96
	1765	-	20	Toxic	Toxic	Toxic	47
	35	+	18	14	19	42	143
	71	+	13	19	20	41	158
	177	+	13	15	19	48	117
	353	+	12	10	21	35	89
	706	+	14	9	23	23	113
	1765	+	11	Toxic	Toxic	Toxic	71
Ozonation Diethylamine	23	-	30	16	13	28	112
	46	-	23	11	17	38	125
	116	-	39	16	16	23	128
	231	-	32	16	25	30	133
	462	-	26	14	12	20	106
	1156	-	25	Toxic	Toxic	23	129
	23	+	26	21	27	28	165
	46	+	15	23	30	34	150
	116	+	15	22	29	38	143
	231	+	19	13	23	44	136
	462	+	17	13	25	33	116
	1156	+	23	Toxic	Contam.	35	131

Table 57

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI

PHENOL

EXPERIMENT 1

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE					
			S. typhimurium					E. coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	35	24	15	15	140	47
		+	8	23	12	14	117	47
Pre-Ozonation Phenol	2500	-	55	16	13	12	126	41
	2500	+	10	12	12	9	121	40
Ozonation Phenol	48	-	56	15	12	12	92	46
	95	-	33	19	18	16	87	23
	238	-	Contam.	12	20	10	106	27
	475	-	80	16	16	21	142	34
	950	-	65	6	12	5	148	32
	2375	-	45	16	18	12	92	31
	48	+	5	16	17	13	133	35
	95	+	6	11	24	11	86	53
	238	+	9	28	15	19	115	39
	475	+	12	14	13	24	120	43
	950	+	11	17	16	24	112	25
	2375	+	15	20	15	14	133	22

Table 58

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

PHENOL

EXPERIMENT 17

Compound	Micrograms of Compound Added Per Plate	MA	HISTIDINE REVERTANTS PER PLATE				
			S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	23	33	18	30	192
		+	17	26	23	41	230
Positive Control							
B-propiolactone	50	-	154				
9 AA	100	-		25			
2-nitrofluorene	50	-			~3000		
AF_2	0.1	-				292	1500
Pre-Ozonation Phenol	3323	-	17	20	14	18	223
	3323	+	12	21	17	25	180
Ozonation Phenol	58	-	19	27	12	20	208
	116	-	19	24	14	19	260
	290	-	17	30	12	23	220
	580	-	1	15	8	17	204
	1160	-	Toxic	14	9	12	121
	2901	-	0	Toxic	20	0	0
	58	+	15	43	20	34	282
	116	+	20	31	12	37	222
	290	+	14	25	19	23	282
	580	+	6	40	25	19	240
	1160	+	4	18	9	22	134
	2901	+	1	1	10	8	7

Table 59

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

HYDROQUINONE

EXPERIMENT 3

Compound	Micrograms of Compound Added per Plate	Activation	REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	35	24	15	15	140
		+	8	23	12	14	117
Pre-Ozonation Hydroquinone	3663	-		2			
	3663	+	16	23	10	18	109
Ozonation Hydroquinone	46	-	43	18	16	14	129
	92	-	39	12	20	15	132
	231	-	35	20	11	12	128
	462	-	46	18	24	14	150
	924	-	48	20	7	10	132
	2311	-	32	12	10	12	105
	46	+	6	21	22	14	112
	92	+	6	26	16	24	114
	231	+	5	22	19	16	110
	462	+	10	24	27	18	128
	924	+	6	15	21	14	139
	2311	+	14	16	19	19	117

Table 60

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
AND ESCHERICHIA COLI

HYDROQUINONE

EXPERIMENT 17

Compound	Micrograms of Compound Added Per Plate	MA	REVERTANTS PER PLATE					
			S. typhimurium					E. Coli
			TA1535	TA1537	TA1538	TA98	TA100	WP2
Negative Control		-	23	33	18	30	192	42
		+	17	26	23	41	230	43
Positive Control								
B-propiolactone	50	-	154					
9 AA	100	-		25				
2-nitrofluorene	50	-			~3000			
AF_2	0.1	-				292	1500	1080
Pre-Ozonation Hydroquinone	2510	-	0	0	Toxic	0	0	3
	2510	+	Toxic	15	Toxic	11	0	19
Ozonation Hydroquinone	38	-	11	25	12	23	198	23
	76	-	12	46	13	22	212	39
	190	-	10	44	8	30	214	35
	381	-	Toxic	20	12	25	162	44
	761	-	Toxic	16	15	15	174	39
	1903	-	0	Toxic	Toxic	Toxic	72	36
	38	+	11	27	14	33	145	50
	76	+	13	30	14	25	164	51
	190	+	9	27	17	27	163	52
	381	+	9	33	19	26	232	38
	761	+	9	31	10	16	168	44
	1903	+	1	6	9	18	80	39

Table 61

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

GLYCEROL

EXPERIMENT 2

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE S. typhimurium				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	36	14	18	21	185
		+	14	16	37	24	197
Pre-Ozonation Glycerol	3000	-	25	13	10	9	157
	3000	+	20	20	17	19	162
Ozonation Glycerol	60	-	15	12	17	15	154
	120	-	34	20	12	18	159
	300	-	18	12	8	9	165
	600	-	25	15	12	22	168
	1200	-	21	10	10	13	153
	3000	-	26	9	15	14	149
	60	+	8	28	15	14	154
	120	+	12	23	15	10	138
	300	+	18	13	10	15	137
	600	+	12	22	15	30	162
	1200	+	10	12	14	Contam.	153
	3000	+	17	10	17	25	131

Table 62

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

GLYCEROL

EXPERIMENT 16

Compound	Compound Added Per Plate	MA	REVERTANTS PER PLATE S. typhimurium TA1535	 TA1537	 TA1538	 TA98	 TA100	 E. Coli WP2
Negative Control		-	59	17	10	40	231	101
		+	45	18	24	54	263	116
Positive Control								
9 AA	100 ug	-		1000				
B-propiolactone	50 ug	-	1000					
Biphenyl	500.5 ug	+			500			
AF_2	.1 ug	-				600	1200	1800
Pre-Ozonation Glycerol	.25 ml	-	65	44	53	38	240	87
	.25 ml	+	58	25	40	48	206	121
Ozonation* Glycerol	.005 ml	-	40	12	22	26	230	71
	.01 ml	-	47	13	10	30	304	92
	.025 ml	-	43	19	10	46	416	105
	.05 ml	-	41	26	10	70	384	117
	.1 ml	-	35	15	20	103	534	142
	.25 ml	-	13	5	15	34	742	280
	.005 ml	+	52	12	14	48	486	98
	.01 ml	+	33	22	11	40	458	82
	.025 ml	+	46	17	18	50	639	110
	.05 ml	+	37	30	19	50	602	118
	.1 ml	+	23	22	23	67	542	147
	.25 ml	+	15	11	16	98	852	175

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*100% of starting material reacted.

Table 63

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

GLYCEROL

EXPERIMENT 28

Compound	Micrograms of Compound Added per Plate	MA	HISTIDINE REVERTANTS PER PLATE			
			S. Typhimurium			
			TA1535	TA1537	TA1538	TA100
Negative Control		-	42	22	45	185
		+	32	22	60	246
Positive Control						
B-propiolactone	50	-	1830			
9 AA	100	-		1500		
1 NF	50	-			1065	
AF_2	0.1	-				3000
2 AN	10	+	2010	1515	6000	7000
Pre-Ozonation Glycerol	2945	-	55	13	19	235
	2945	+	32	23	39	260
Ozonation Glycerol	54	-	51	12	20	268
	109	-	51	23	11	288
	273	-	78	16	22	288
	545	-	86	17	28	260
	1090	-	63	14	43	280
	2724	-	74	14	25	302
	54	+	32	21	31	224
	109	+	52	19	36	320
	273	+	30	19	42	260
	545	+	33	27	35	276
	1090	+	35	23	45	230
	2724	+	33	22	51	293

Table 64

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

ALANYLGLYCYLGLYCINE

EXPERIMENT 28

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Negative Control		-	56	Contam.	Contam.	11	229
		+	22	Contam.	Contam.	18	239
Positive Control							
B-Propiolactone		-	1200	Contam.	Contam.		
9AA		-		Contam.	Contam.		
2NF		-		Contam.	Contam.		
AF2		-		Contam.	Contam.	17	1000
2AN		+	600	Contam.	Contam.	77	2500
Pre-Ozonation Alanylglycylglycine	116	-	20	Contam.	Contam.	17	223
	116	-	16	Contam.	Contam.	18	184
Ozonation Alanylglycylglycine	2.3	-	6	Contam.	Contam.	26	187
	4.6	-	12	Contam.	Contam.	16	206
	11.6	-	13	Contam.	Contam.	21	202
	23.1	-	21	Contam.	Contam.	10	197
	46.2	-	20	Contam.	Contam.	13	200
	115.5	-	16	Contam.	Contam.	11	179
	2.3	+	24	Contam.	Contam.	23	191
	4.6	+	28	Contam.	Contam.	20	160
	11.6	+	28	Contam.	Contam.	18	154
	23.1	+	40	Contam.	Contam.	22	172
	46.2	+	Contam.	Contam.	Contam.	26	218
	115.5	+	20			9	169

Table 65

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

ALANYLGLYCYLGLYCINE

EXPERIMENT 38

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE *S. typhimurium* TA1537	REVERTANTS PER PLATE *S. typhimurium* TA1538
Negative Control		-	23	33
		+	32	52
Pre-Ozonation Alanylglycylglycine	8.4	-	14	23
	8.4	+	35	50
Ozonation Alanylglycylglycine	1.7	-	18	33
	3.4	-	19	18
	5.0	-	20	42
	6.7	-	17	26
	8.4	-	17	27
	1.7	+	28	53
	3.4	+	20	36
	5.0	+	30	30
	6.7	+	20	43
	8.4	+	24	36

Table 66

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

ALANYLGLYCYLGLYCINE

EXPERIMENT 40

Compound	Micrograms of Compound Added per Plate	Metabolic Activation	REVERTANTS PER PLATE *S. typhimurium*				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
		-	17	11	16	25	133
		+	11	16	17	37	134
Positive Control							
B-Propialactone		-	802				
9AA		-		1122			
2NF		-			1853		
AF2		-				250	1111
2AN		-	18	9	13	34	154
2AN		+	60	24	266	285	609
Preozonation	8.4	-	18	7	9	25	127
Alanylglycylglycine	8.4	+	11	7	25	46	130
Ozonation	1.7	-	23	15	13	24	141
Alanylglycylglycine	3.4	-	9	12	9	27	139
	5.0	-	19	12	9	22	123
	6.7	-	18	15	13	27	139
	8.4	-	19	8	5	36	132
	1.7	+	6	5	15	35	150
	3.4	+	11	14	17	30	137
	5.0	+	8	11	19	42	106
	6.7	+	9	8	14	30	123
	8.4	+	19	15	29	34	130

Table 67

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH 2,4-DINITROTOLUENE

EXPERIMENT 5

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	3.5	5.6
	+		5.4	100	3.5	6.5
Pre-Ozonation	-	.008	6.0	95	2.0	3.3
	+	.008	4.5	83	5.0	11.1
Ozonated	-	.001	8.4	133	3.0	3.6
	-	.0005	7.1	113	2.5	3.5
	-	.00025	6.6	105	2.0	3.0
	-	.0001	5.8	92	1.0	1.7
	-	.00002	4.9	78	1.3	2.7
	+	.001	Contam.	Contam.	Contam.	Contam.
	+	.0005	7.0	130	3.8	5.4
	+	.00025	6.9	128	2.0	2.9
	+	.0001	6.5	120	5.0	7.7
	+	.00002	5.6	104	3.3	5.9

Table 68

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH 2,4-DINITROTOLUENE

EXPERIMENT 15

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.8	100	4.5	6.7
	+		6.9	100	3.5	5.1
Pre-Ozonation	-	.003	8.0	118	5.0	6.2
	+	.003	6.5	95	13.0	20.0
Ozonated	-	.001	5.9	86	8.0	13.6
	-	.0005	6.2	91	2.5	4.1
	-	.00025	16.0	234	7.5	4.7
	-	.0001	4.4	65	12.0	27.3
	-	.00002	4.5	66	17.0	37.8
	+	.001	4.4	63	6.0	13.6
	+	.0005	4.3	63	6.3	14.7
	+	.00025	5.2	76	5.0	9.6
	+	.0001	3.4	49	7.0	20.6
	+	.00002	4.8	70	5.0	10.4

Table 69

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZOIC ACID

EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.8	100	6.0	10.3
	+		6.2	100	6.0	9.7
Pre-Ozonation	-	.40	5.9	101	7.0	11.9
	+	.40	5.3	85	2.0	3.8
Ozonated	-	.15	7.6	131	3.0	3.9
	-	.075	7.1	122	7.0	9.9
	-	.04	6.8	117	5.0	7.4
	-	.015	4.3	74	6.0	14.0
	-	.003	8.7	150	8.0	9.2
	+	.15	4.7	76	<1.0	2.1
	+	.075	5.6	90	7.0	12.5
	+	.04	8.2	132	5.0	6.1
	+	.015	9.8	158	3.0	3.1
	+	.003	7.2	116	3.0	4.2

Table 70

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZOIC ACID

EXPERIMENT 15

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.8	100	4.5	6.7
	+		6.9	100	3.5	5.1
Pre-Ozonation	-	.14	10.8	159	5.0	4.6
	+	.14	9.5	139	3.0	3.2
Ozonated	-	.12	12.6	184	15.0	11.5
	-	.06	plating error		plating error	
	-	.03	plating error		plating error	
	-	.012	6.3	93	10.0	15.9
	-	.002	7.7	113	12.0	15.6
	+	.12	10.5	154	9.0	8.5
	+	.06	plating error		plating error	
	+	.03	11.3	165	8.0	7.1
	+	.012	6.3	92	7.0	11.1
	+	.002	9.9	145	7.0	7.1

Table 71

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ETHANOL

EXPERIMENT 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		8.9	100	4.9	5.5
	+		8.4	100	6.3	7.5
Pre-Ozonation	-	.31	15.1	169	6.0	4.0
	+	.31	13.1	146	6.0	4.6
Ozonated (10 min.)	-	.23	11.2	124	7.5	6.8
	-	.12	10.4	112	8.0	8.0
	-	.06	11.5	135	6.0	5.0
	-	.02	9.0	101	2.5	2.8
	-	.005	9.7	109	1.0	1.0
	+	.23	10.2	119	5.0	5.0
	+	.12	11.1	131	8.0	7.3
	+	.06	11.7	143	13.0	11.1
	+	.02	9.4	112	6.0	6.4
	+	.005	10.2	119	7.0	7.0

Table 71a

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ETHANOL

EXPERIMENT 1

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		8.9	100	4.9	5.5
	+		8.4	100	6.3	7.5
Ozonated (4 hrs.)	-	.23	9.7	109	118.0	121.6
	-	.12	9.4	106	33.0	35.1
	-	.06	9.0	101	13.0	14.4
	-	.023	8.5	96	8.0	9.4
	-	.005	8.2	92	3.0	3.7
	+	.23	8.3	99	13.0	15.7
	+	.12	9.0	107	6.0	6.7
	+	.06	9.7	115	7.5	7.7
	+	.023	8.6	102	5.0	5.8
	+	.005	7.7	92	4.0	5.2

Table 72

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ETHANOL

EXPERIMENT 16

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.1	100	<1.0	2.5
	+		3.7	100	3.5	9.5
Pre-Ozonation	-	.31	4.2	103	4.0	9.5
	-	.31	4.2	114	3.0	7.1
Ozonated	-	.08	9.7	237	52.0	53.6
	-	.04	3.1	75	293.0	945.2
	-	.02	3.0	73	157.0	523.3
	-	.008	3.8	92	3.0	8.0
	-	.0016	4.3	106	<1.0	2.3
	+	.08	3.2	87	90.0	281.3
	+	.04	3.4	93	21.0	61.8
	+	.02	3.5	95	3.0	8.6
	+	.008	4.6	124	<1.0	2.2
	+	.0016	4.0	108	1.0	2.5

Table 73

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 WITH ETHANOL

EXPERIMENT 37

Compound (old sample)	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.1	100	3.0	4.2
	+		6.8	100	2.0	3.0
Ozonated	-	.08	6.8	96	182.0	267.0
	-	.04	5.0	70	50.0	100.0
	-	.02	5.7	80	38.0	67.0
	-	.008	7.1	100	16.0	22.6
	-	.0016	7.2	101	13.0	18.1
	+	.08	6.0	88	9.0	15.1
	+	.04	7.2	106	5.0	6.9
	+	.02	5.9	87	15.0	25.3
	+	.008	6.3	93	5.0	7.9
	+	.0016	7.1	104	11.0	15.6

Table 74

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZO(a)PYRENE

EXPERIMENT 13

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negtive Control	-		8.3	100	2.5	3.0
	+		7.6	100	3.5	4.6
Pre-Ozonation	-	1 ml	9.6	116	3.0	3.1
(saturated solution)	+	1 ml	8.5	112	5.0	5.9
Ozonated	-	1 ml	8.5	102	4.0	4.7
(saturated solution)	-	.5 ml	8.8	107	3.0	3.4
	-	.25 ml	8.1	98	4.0	5.0
	-	.1 ml	9.6	116	1.0	1.0
	-	.02 ml	9.0	108	3.0	3.3
	+	1 ml	8.0	106	2.0	2.5
	+	.5 ml	7.9	105	5.0	6.3
	+	.25 ml	8.1	108	12.0	14.8
	+	.1 ml	7.7	101	2.0	2.6
	+	.02 ml	7.8	104	4.0	5.1

Table 75

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 WITH BENZO(a)PYRENE

EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.9	100	1.0	1.3
	+		12.0	100	8.0	6.9
Pre-Ozonation	-	2×10^{-8}	7.6	96	8.0	10.5
	+	2×10^{-8}	7.4	64	6.0	8.1
Ozonated*	-	1 ml	7.6	97	3.0	3.9
	-	.5 ml	8.2	104	10.0	12.2
	-	.25 ml	7.8	98	8.0	10.3
	-	.1 ml	6.4	81	1.3	1.9
	-	.02 ml	6.2	79	7.0	11.3
	+	1 ml	7.9	69	6.0	7.6
	+	.5 ml	7.9	68	10.0	12.7
	+	.25 ml	6.7	58	4.0	6.0
	+	.1 ml	6.2	54	7.0	11.3
	+	.02 ml	7.8	67	6.0	7.7

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*100% of starting material reacted.

Table 76

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HMX

EXPERIMENT 27

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.9	100	1.0	1.3
	+		12.0	100	8.0	6.9
Pre-Ozonation	-	.000012				
	+	.000012	6.9	60	7.0	10.1
Ozonated	-	.000006	5.8	74	8.0	13.8
	-	.000003	7.4	94	11.0	14.9
	-	.0000015	6.4	80	2.0	3.2
	-	.0000006	6.0	76	4.0	6.7
	-	.00000012	8.6	109	10.0	11.6
	+	.000006	7.0	60	10.0	14.3
	+	.000003	7.0	61	7.0	10.0
	+	.0000015	5.5	48	13.0	23.6
	+	.0000006	6.1	53	5.0	8.2
	+	.00000012	7.0	61	4.0	5.7

Table 77

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HMX

EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	5.5	1.7
	+		3.5	100	4.0	11.3
Pre-Ozonation	-	.000011	3.1	97	7.0	22.6
	+	.000011	2.8	80	6.0	21.4
Ozonated	-	.0000066	4.0	125	7.0	17.5
	-	.0000033	3.6	113	3.0	8.3
	-	.0000017	4.9	153	6.0	12.2
	-	.00000066	5.2	163	13.0	25.0
	-	.00000013	16.2	506	11.0	6.8
	+	.0000066	3.0	86	4.0	13.3
	+	.0000033	4.0	114	7.0	17.5
	+	.0000017	3.3	94	2.0	6.1
	+	.00000066	8.4	240	4.0	4.8
	+	.00000013	4.4	126	9.0	20.5

Table 78

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH RDX

EXPERIMENT 26

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.5	100	4.0	7.3
	+		6.0	100	6.0	10.0
Pre-Ozonation	-	.001	6.8	124	7.0	10.3
	+	.001	5.8	96	5.0	8.6
Ozonated*	-	1 ml	6.9	125	6.0	8.7
	-	.5 ml	7.4	136	6.0	8.1
	-	.25 ml	9.3	169	3.0	3.2
	-	.1 ml	6.4	116	5.0	7.9
	-	.02 ml	5.5	101	8.0	14.5
	+	1 ml	7.3	121	7.0	9.6
	+	.5 ml	6.6	109	9.0	13.6
	+	.25 ml	6.9	114	6.0	8.7
	+	.1 ml	5.1	85	3.0	5.9
	+	.02 ml	6.4	106	9.0	14.1

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*% reacted unknown.

Table 79

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH RDX

EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	5.5	1.7
	+		3.5	100	4.0	11.3
Pre-Ozonation	-	.001	3.8	119	1.7	4.5
	+	.001	3.6	103	6.0	16.7
Ozonated	-	.0009	4.3	134	4.0	9.3
	-	.00045	3.5	109	2.0	5.8
	-	.000125	7.1	222	4.0	5.6
	-	.00009	4.1	128	5.0	12.3
	-	.000018	3.5	109	4.0	11.4
	+	.0009	2.9	83	2.0	6.7
	+	.00045	3.1	89	2.0	6.4
	+	.000125	3.6	103	3.0	8.4
	+	.00009	4.2	120	5.0	11.9
	+	.000018	2.4	69	5.0	21.3

Table 80

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 WITH OLEIC ACID

EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($x\ 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($x\ 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.1	100	3.9	5.5
	+		8.2	100	8.5	10.4
Pre-Ozonation (saturated solution)	-	1 ml	6.4	91	3.0	4.7
	+	1 ml	6.3	77	5.0	8.0
Ozonated (saturated solution)	-	1 ml	6.6	92	7.0	10.6
	-	.5 ml	6.4	90	7.0	10.9
	-	.25 ml	7.7	109	4.0	5.2
	-	.1 ml	7.4	104	6.0	8.1
	-	.02 ml	7.0	98	7.0	10.0
	+	1 ml	7.6	93	7.0	9.3
	+	.5 ml	5.1	63	9.0	17.6
	+	.25 ml	6.2	76	5.0	8.0
	+	.1 ml	6.3	78	13.0	20.6
	+	.02 ml	7.4	91	6.0	8.1

Table 81

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH OLEIC ACID

EXPERIMENT 25

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		8.8	100	2.5	2.9
	+		5.5	100	5.5	10.0
Pre-Ozonation	-	0.004	5.8	66	7.0	12.1
	+	0.004	5.8	106	6.0	10.3
Ozonated*	-	1 ml	3.5	40	5.0	14.3
	-	.5 ml	4.7	53	5.0	10.6
	-	.25 ml	5.5	63	2.0	3.6
	-	.1 ml	5.4	61	4.0	7.5
	-	.02 ml	5.3	61	7.0	13.2
	+	1 ml	5.9	108	5.0	8.5
	+	.5 ml	4.3	79	6.0	14.0
	+	.25 ml	4.8	89	5.0	10.4
	+	.1 ml	4.7	86	7.0	14.9
	+	.02 ml	5.2	94	7.0	13.5

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*100% of starting material reacted.

Table 82

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HUMIC ACIDS

EXPERIMENT 14

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Recombinants per ml (x 10^{-3})	Recombinants per 10^5 Survivors
Negative Control	-		7.1	100	3.9	5.5
	+		8.2	100	8.5	10.4
Pre-Ozonation (saturated solution)	-	1 ml	7.3	103	6.0	8.2
	+	1 ml	7.2	89	10.0	13.9
Ozonated (saturated solution)	-	1 ml	7.5	106	5.0	6.7
	-	.5 ml	6.8	96	4.0	5.9
	-	.25 ml	8.4	118	2.0	2.4
	-	.1 ml	7.3	103	9.0	12.3
	-	.02 ml	6.9	98	7.0	10.1
	+	1 ml	7.5	92	8.0	10.7
	+	.5 ml	7.0	86	3.0	4.3
	+	.25 ml	8.0	98	2.0	2.5
	+	.1 ml	6.4	79	9.0	14.1
	+	.02 ml	6.9	85	7.0	10.1

Table 83

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HUMIC ACIDS

EXPERIMENT 25

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		8.8	100	2.5	2.9
	+		5.5	100	5.5	10.0
Pre-Ozonation (saturated solution)	-	1 ml	6.3	72	4.0	6.3
	+	1 ml	5.8	107	2.0	3.4
Ozonated	-	1 ml	6.1	69	4.0	6.6
	-	.5	5.6	64	9.0	16.1
	-	.25	7.7	88	6.0	7.8
	-	.1	5.0	57	5.0	10.0
	-	.02	4.8	55	5.0	10.4
	+	1 ml	6.0	110	3.0	5.0
	+	.5	6.6	122	3.0	4.5
	+	.25	4.5	82	2.0	4.5
	+	.1	4.0	73	3.0	7.5
	+	.02	4.6	85	2.0	4.3

Table 84

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HUMIC ACIDS

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.6	100	10.0	14.8
	+		6.1	100	6.5	10.6
Pre-Ozonation (saturated solution)	-	1 ml	6.8	103	10.0	14.6
	+	1 ml	7.0	115	7.0	10.1
Ozonated (saturated solution)	-	1 ml	6.3	95	5.0	8.0
	-	.5 ml	6.4	97	7.0	10.9
	-	.25 ml	6.8	103	8.0	11.7
	-	.1 ml	8.2	125	7.0	8.6
	-	.02 ml	7.8	118	1.0	1.3
	+	1 ml	5.8	95	13.0	2.2
	+	.5 ml	6.4	105	11.0	17.1
	+	.25 ml	6.6	108	11.0	16.7
	+	.1 ml	6.7	110	9.0	13.5
	+	.02 ml	6.3	97	10.0	15.8

Table 85

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH TNT CONDENSATE WATER

EXPERIMENT 11

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($x 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($x 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		2.6	100	3.5	13.5
	+		1.9	100	3.5	18.4
Pre-Ozonation	-	.002	2.2	84	5.0	22.7
	+	.002	2.7	140	4.0	14.8
Ozonated	-	.0016	2.5	92	5.0	20.0
	-	.0008	1.9	71	5.0	26.3
	-	.0004	2.1	82	4.0	19.0
	-	.00016	2.0	75	2.0	10.0
	-	.000032	1.6	63	5.0	31.3
	+	.0016	2.3	118	5.0	21.7
	+	.0008	2.2	112	6.0	27.3
	+	.0004	2.0	105	4.0	20.0
	+	.00016	2.5	131	4.0	16.0
	+	.000032	1.0	49	2.0	20.0

Table 86

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH TNT CONDENSATE WATER

EXPERIMENT 24

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.1	100	3.5	5.7
	+		5.7	100	4.5	7.9
Pre-Ozonation	-	0.0014	5.4	88	6.0	11.1
	+	0.0014	3.9	68	3.0	7.8
Ozonated	-	0.0012	4.4	72	5.0	11.4
	-	0.0006	3.9	63	2.0	5.2
	-	0.0003	4.9	80	8.0	16.3
	-	0.00012	6.8	111	3.8	5.5
	-	0.000024	7.6	125	13.0	17.1
	+	0.0012	4.3	75	3.0	6.9
	+	0.0006	3.6	62	7.0	19.4
	+	0.0003	5.1	88	2.0	4.0
	+	0.00012	6.4	111	7.0	10.9
	+	0.000024	6.4	112	8.0	12.5

Table 87

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIPHENYLHYDRAZINE

EXPERIMENT 26

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.5	100	4.0	7.3
	+		6.0	100	6.0	10.0
Pre-Ozonation	-	.33	3.7	68	8.0	21.6
	+	.33	3.1	52	2.0	6.5
Ozonation	-	.22	1.6	29	10.0	62.5
	-	.11	1.7	31	6.0	35.3
	-	.055	7.2	131	12.0	16.7
	-	.022	9.3	169	8.0	8.6
	-	.0044	5.1	93	5.0	9.8
	+	.22	5.9	98	4.0	6.8
	+	.11	2.8	46	11.0	39.3
	+	.055	4.2	70	5.0	11.9
	+	.022	4.6	76	10.0	21.7
	+	.0044	6.3	104	3.0	4.8

Table 88

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIPHENYLHYDRAZINE

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.2
	+		8.7	100	5.0	5.0
Pre-Ozonation	-	.18	6.7	92	4.0	6.0
	+	.18	Contam.	Contam.	Contam.	Contam.
Ozonation	-	.04	5.1	70	7.0	13.7
	-	.02	6.4	88	6.0	9.4
	-	.01	Contam.	Contam.	Contam.	Contam.
	-	.004	8.0	110	5.0	6.3
	-	.0008	6.7	92	5.0	7.5
	+	.04	Contam.	Contam.	Contam.	Contam.
	+	.02	6.5	75	10.0	15.4
	+	.01	7.7	89	4.0	5.2
	+	.004	6.2	71	3.0	4.8
	+	.0008	Contam.	Contam.	Contam.	Contam.

Table 89

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIPHENYLHYDRAZINE

EXPERIMENT 34

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.0	100	5.0	7.1
	+		7.0	100	5.5	7.9
Ozonation	-	.0032	11.0	157	6.0	5.5
	-	.0016	7.2	103	7.0	9.7
	-	.0008	5.2	74	8.0	15.4
	-	.0003	16.0	229	7.0	4.4
	-	.00006	9.9	141	4.0	4.0
	+	.0032	14.0	200	10.0	7.1
	+	.0016	5.6	80	9.0	16.1
	+	.0008	5.2	74	7.0	13.5
	+	.0003	7.0	100	10.0	14.3
	+	.00006	6.5	93	5.0	7.7

Table 90

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH NITRILIOACETIC ACID

EXPERIMENT 13

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		8.3	100	2.5	3.0
	+		7.6	100	3.5	4.6
Pre-Ozonation	-	.4	6.7	81	4.0	6.0
	+	.4	8.0	106	3.0	3.8
Ozonated	-	.17	7.8	94	5.0	6.4
	-	.09	8.1	98	3.0	3.7
	-	.04	7.9	96	4.0	5.0
	-	.02	8.1	98	7.0	8.6
	-	.004	7.3	88	4.0	5.5
	+	.17	7.1	93	7.0	9.9
	+	.09	9.0	119	3.0	3.4
	+	.04	8.4	111	5.0	6.0
	+	.02	8.3	109	5.0	6.1
	+	.004	5.4	71	10.0	18.5

Table 91

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH NITRILIOACETIC ACID

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	2.0	3.2
	+		7.0	100	1.0	1.4
Pre-Ozonation	-	.42	7.1	112	5.0	7.0
	+	.42	6.0	85	1.0	1.7
Ozonated	-	.21				
	-	.11	5.2	82	4.0	7.7
	-	.05	5.0	80	1.0	2.0
	-	.02	4.9	78	3.0	6.1
	-	.004	4.8	76	4.0	8.3
	+	.21	5.9	84	11.0	18.6
	+	.11	5.1	72	8.0	15.7
	+	.05	7.4	104	1.3	1.7
	+	.02	5.1	72	4.0	7.9
	+	.004	5.1	73	1.7	3.3

Table 92

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH NITRILIOACETIC ACID

EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	5.5	17.0
	+		3.5	100	4.0	11.3
Pre-Ozonation	-	.54	4.0	125	4.0	10.0
	-	.27	4.9	153	1.0	2.1
	-	.13	3.5	100	5.0	14.3
	-	.05	5.7	178	3.0	5.2
	-	.01	4.1	128	9.0	21.8
	+	.54	4.0	114	2.0	5.1
	+	.27	3.0	86	7.0	23.7
	+	.13	3.1	86	3.0	9.7
	+	.05	4.4	126	2.0	4.6
	+	.01	4.6	131	5.0	10.9
Ozonated	-	.27	4.6	144	11.0	24.0
	-	.13	5.1	159	2.0	3.9
	-	.07	4.6	144	3.0	6.5
	-	.03	4.2	131	6.0	14.4
	-	.005	4.8	150	7.0	14.5
	+	.27	4.5	129	14.0	30.9
	+	.13	5.0	143	5.0	10.1
	+	.07	5.1	146	8.0	15.8
	+	.03	4.7	134	4.0	8.5
	+	.005	5.1	146	5.0	9.8

Table 93

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ALDRIN

EXPERIMENT 12

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.8	100	5.5	8.1
	+		6.6	100	10.5	15.9
Pre-Ozonation (saturated solution)	-	1 ml	6.2	90	3.0	4.9
	+	1 ml	6.0	91	5.0	8.3
Ozonated (saturated solution)	-	1 ml	6.8	100	13.0	19.1
	-	.5 ml	6.5	96	13.0	20.0
	-	.25 ml	7.0	103	14.0	20.0
	-	.1 ml	6.4	93	11.0	17.2
	-	.02 ml	6.3	93	14.0	22.2
	+	1 ml	5.9	89	5.0	8.5
	+	.5 ml	7.3	111	8.0	11.0
	+	.25 ml	6.4	97	11.0	17.2
	+	.1 ml	6.9	104	9.0	13.0
	+	.02 ml	5.2	79	6.0	11.5

Table 94

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ALDRIN

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	2.0	3.2
	+		7.0	100	1.0	1.4
Pre-Ozonation (saturated solution)	-	1 ml	7.2	113	3.8	5.2
	+	1 ml	7.3	103	8.8	12.1
Ozonated (saturated solution)	-	1 ml	6.1	96	3.0	5.0
	-	.5 ml	8.3	132	3.0	3.6
	-	.25 ml	8.0	126	3.0	3.8
	-	.1 ml	6.4	101	1.0	1.6
	-	.02 ml	8.0	127	6.3	7.8
	+	1 ml	5.6	80	7.0	12.5
	+	.5 ml	8.0	114	2.0	2.5
	+	.25 ml	7.6	108	6.0	7.9
	+	.1 ml	6.7	95	1.0	1.5
	+	.02 ml	6.6	93	5.0	7.6

Table 95

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DDT

EXPERIMENT 12

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.8	100	5.5	8.1
	+		6.6	100	10.5	15.9
Pre-Ozonation (saturated solution)	-	1 ml	5.9	86	9.0	15.3
	+	1 ml	6.3	95	8.0	12.7
Ozonated (saturated solution)	-	1 ml	7.8	114	8.0	10.3
	-	.5 ml	7.5	110	4.0	5.4
	-	.25 ml	6.9	102	8.0	11.6
	-	.1 ml	8.2	119	9.0	11.0
	-	.02 ml	6.8	99	7.0	10.3
	+	1 ml	8.4	127	8.0	9.5
	+	.5 ml	8.1	122	8.0	9.9
	+	.25 ml	6.9	105	11.0	15.9
	+	.1 ml	Contam.	Contam.	Contam.	Contam.
	+	.02 ml	6.8	103	10.0	14.7

Table 96

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DDT

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	2.0	3.2
	+		7.0	100	1.0	1.4
Ozonated (saturated solution)	-	1 ml	8.3	132	3.0	3.6
	-	.5 ml	9.3	147	1.0	1.1
	-	.25 ml	7.5	119	2.0	2.7
	-	.1 ml	7.1	112	4.0	5.7
	-	.02 ml	6.0	95	2.0	3.3
	+	1 ml	8.4	133	1.0	1.2
	+	.5 ml	7.6	108	1.0	1.3
	+	.25 ml	7.4	105	2.0	2.7
	+	.1 ml	6.9	98	3.0	4.4
	+	.02 ml	6.7	96	1.3	1.9

Table 97

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH AROCHLOR

EXPERIMENT 10

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.8	100	4.3	5.5
	+		5.9	100	7.5	13.0
Pre-Ozonation (saturated solution)	-	1 ml	6.1	78	5.0	8.2
	+	1 ml	7.3	123	3.0	4.1
Ozonated (saturated solution)	-	1 ml	6.8	87	8.0	11.8
	-	.5 ml	7.6	97	9.0	11.8
	-	.25 ml	6.5	83	9.0	13.8
	-	.1 ml	7.3	93	6.0	8.2
	-	.02 ml	6.2	80	5.0	8.0
	+	1 ml	6.2	104	5.0	8.1
	+	.5 ml	6.2	105	6.0	9.7
	+	.25 ml	6.9	117	3.8	5.4
	+	.1 ml	7.7	130	8.0	10.4
	+	.02 ml	6.4	107	1.0	1.6

Table 98

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH AROCHLOR

EXPERIMENT 23

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	2.0	3.2
	+		7.0	100	1.0	1.4
Pre-Ozonation (saturated solution)	-	1 ml	7.9	124	4.0	5.1
	+	1 ml	7.6	108	1.0	1.3
Ozonated (saturated solution)	-	1 ml	9.3	147	2.0	2.2
	-	.5 ml	8.5	134	4.0	4.7
	-	.25 ml	7.3	115	2.0	2.8
	-	.1 ml	7.8	124	2.0	2.6
	-	.02 ml	8.2	129	5.0	6.1
	+	1 ml	7.7	110	3.0	3.9
	+	.5 ml	7.3	104	4.0	5.5
	+	.25 ml	7.8	111	4.0	5.1
	+	.1 ml	7.9	112	1.0	1.3
	+	.02 ml	7.4	105	4.0	5.4

Table 99

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ACETIC ACID

EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.2
	+		9.9	100	4.5	4.5
Pre-Ozonation	-	.40	7.1	97	2.0	2.8
	+	.40	6.3	64	3.0	4.8
Ozonated	-	.40	3.9	53	4.0	10.3
	+	.40	7.0	71	5.0	7.1

Table 100

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH ACETIC ACID

EXPERIMENT 22

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	−		5.2	100	4.0	7.6
	+		4.9	100	2.5	5.1
Pre-Ozonation	−	.50	8.7	167	3.0	3.5
	+	.50	7.6	156	2.0	2.6
Ozonated	−	.48	7.3	140	2.0	2.7
	−	.24	7.3	140	1.0	1.4
	−	.12	7.5	145	3.0	4.0
	−	.048	6.4	124	4.0	6.2
	−	.0096	6.3	121	2.0	3.2
	+	.48	7.9	163	1.0	1.3
	+	.24	7.6	157	4.0	5.3
	+	.12	5.0	103	1.0	2.0
	+	.048	7.7	159	4.0	5.2
	+	.0096	8.5	173	3.0	3.6

Table 101

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLUCOSE

EXPERIMENT 10

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.8	100	4.3	5.5
	+		5.9	100	7.5	12.7
Pre-Ozonation	-	.4	7.6	97	8.0	10.5
	+	.4	7.7	130	9.0	11.7
Ozonated*	-	1 ml	8.4	107	5.0	6.0
	-	.5 ml	7.0	89	5.0	7.2
	-	.25 ml	7.3	94	3.0	4.1
	-	.1 ml	7.1	90	2.0	2.8
	-	.02 ml	7.7	99	5.0	6.5
	+	1 ml	6.9	116	4.0	5.8
	+	.5 ml	7.7	130	5.0	6.5
	+	.25 ml	7.4	124	5.0	6.8
	+	.1 ml	6.3	106	7.0	11.1
	+	.02 ml	6.1	103	3.0	4.9

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*100% of starting material reacted.

Table 102

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLUCOSE

EXPERIMENT 21

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		9.9	100	5.0	5.1
	+		9.1	100	4.5	4.6
Pre-Ozonation	-	1 ml	5.3	53	2.0	3.8
	+	1 ml	5.3	59	6.0	11.0
Ozonated*	-	1 ml	5.1	51	3.0	5.9
	-	.5 ml	5.3	53	3.0	5.7
	-	.25 ml	3.6	37	7.0	19.4
	-	.1 ml	4.8	49	3.0	6.2
	-	.02 ml	5.4	55	1.0	1.9
	+	1 ml	4.9	54	5.0	10.2
	+	.5 ml	5.4	60	4.0	7.4
	+	.25 ml	4.1	46	5.0	12.2
	+	.1 ml	5.0	55	4.0	8.0
	+	.02 ml	5.3	58	3.0	5.7

---

*100% of starting material reacted.

Table 103

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH UREA

EXPERIMENT 9

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($x\ 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($x\ 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.2	100	6.0	9.7
	+		5.9	100	5.0	8.5
Pre-Ozonation	-	.4	5.7	92	4.0	7.0
	+	.4	4.1	69	6.0	14.6
Ozonated	-	.4	5.4	87	5.0	9.3
	-	.2	4.1	66	3.0	7.3
	-	.1	6.2	100	1.0	1.6
	-	.04	4.5	73	5.0	11.1
	-	.008	5.2	84	3.0	5.8
	+	.4	5.3	90	5.0	9.4
	+	.2	5.4	92	1.0	1.9
	+	.1	5.4	87	8.0	14.8
	+	.04	5.4	92	4.0	7.4
	+	.008	7.7	131	5.0	6.5

Table 104

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH UREA

EXPERIMENT 21

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		9.9	100	5.0	5.1
	+		9.1	100	4.5	4.6
Pre-Ozonation	-	.38	7.5	76	2.0	2.7
	+	.38	6.4	70	7.0	10.9
Ozonated	-	.36	9.1	92	3.0	3.3
	-	.18	6.4	65	6.0	9.4
	-	.09	7.5	75	5.0	6.7
	-	.036	7.4	75	4.0	5.4
	-	.007	6.4	65	4.0	6.2
	+	.36	7.5	83	2.0	2.7
	+	.18	5.5	60	2.0	3.7
	+	.09	7.2	79	4.0	5.6
	+	.036	8.2	90	9.0	11.0
	+	.007	6.2	68	1.0	1.6

Table 105

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CHOLESTEROL

EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.0	100	3.5	5.0
	+		5.8	100	5.0	8.6
Pre-Ozonation (saturated solution)	-	1 ml	wrong dilution plated			
	+	1 ml	wrong dilution plated			
Ozonated (saturated solution)	-	1 ml	7.1	101	2.5	3.5
	-	.5 ml	6.7	96	2.0	3.0
	-	.25 ml	5.9	84	4.0	6.8
	-	.1 ml	5.6	80	5.0	8.9
	-	.02 ml	6.2	89	2.0	3.2
	+	1 ml	6.3	109	1.3	2.1
	+	.5 ml	4.4	76	4.0	9.1
	+	.25 ml	5.8	100	5.0	8.6
	+	.1 ml	Contam.	Contam.	4.0	Contam.
	+	.02 ml	Contam.	Contam.	6.0	Contam.

Table 106

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CHOLESTEROL

EXPERIMENT 20

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.7	100	1.0	2.2
	+		4.9	100	3.0	6.1
Pre-Ozonation (saturated solution)	-	1 ml	9.7	207	6.0	6.2
	+	1 ml	10.8	219	3.0	4.7
Ozonated (saturated solution)	-	1 ml	10.1	216	4.0	3.9
	-	.5 ml	Contam.	Contam.	Contam.	Contam.
	-	.25 ml	18.9	402	1.0	5.3
	-	.1 ml	10.5	222	6.0	5.7
	-	.02 ml	8.0	170	1.0	1.3
	+	1 ml	7.8	160	1.0	1.3
	+	.5 ml	Contam.	Contam.	Contam.	Contam.
	+	.25 ml	9.2	188	5.0	5.4
	+	.1 ml	16.3	333	1.0	0.61
	+	.02 ml	7.2	147	1.0	1.4

Table 107

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZIDINE

EXPERIMENT 9

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10-7)	Survivors (%)	Mitotic Recombinants per ml (x 10-3)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.2	100	6.0	9.7
	+		5.9	100	5.0	8.5
Pre-Ozonation	-	.2	6.3	102	5.0	7.9
	+	.2	5.8	98	6.0	10.3
Ozonated	-	.08	4.5	73	61.0	135.5
	-	.04	6.6	106	9.0	13.6
	-	.02	5.7	92	2.0	3.5
	-	.008	6.1	98	3.0	4.9
	-	.0016	4.7	76	1.0	2.1
	+	.08	5.2	88	29.0	55.8
	+	.04	5.9	100	6.0	10.2
	+	.02	5.8	98	7.0	12.1
	+	.008	4.4	8	1.0	2.3
	+	.0016	3.8	64	8.0	21.1

Table 108

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZIDINE

EXPERIMENT 20

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10-7)	Survivors (%)	Mitotic Recombinants per ml (x 10-3)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.7	100	1.0	2.2
	+		4.9	100	3.0	6.1
Pre-Ozonation	-	.19	7.4	158	1.0	1.4
	+	.19	7.2	147	4.0	5.5
Ozonated	-	.068	6.3	133	26.0	41.3
	-	.034	4.3	90	2.0	4.7
	-	.017	4.9	104	4.0	8.2
	-	.0068	5.2	111	5.0	9.6
	-	.0014	5.2	110	2.0	3.9
	+	.068	3.6	73	6.0	16.7
	+	.034	4.8	97	1.3	2.6
	+	.017	4.5	92	2.0	4.4
	+	.0068	3.5	72	1.0	2.8
	+	.0014	4.1	83	4.0	9.8

Table 109

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZIDINE

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.3
	+		8.7	100	4.5	5.0
Pre-Ozonation	-	.13	8.9	122	12.0	13.5
	+	.13	4.9	56	2.0	4.1
Ozonated	-	.014	7.1	97	7.0	9.9
	-	.0105	8.6	118	9.0	3.5
	-	.007	7.1	97	11.0	15.5
	-	.0035	6.0	82	12.0	20.0
	-	.0014	6.9	95	7.0	10.1
	+	.014	5.8	67	5.0	8.6
	+	.0105	5.7	66	4.0	7.0
	+	.007	6.3	72	6.0	9.5
	+	.0035	5.3	61	6.0	11.3
	+	.0014	5.9	68	3.0	5.1

Table 110

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZIDINE

EXPERIMENT 36

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.0	100	5.5	11.0
	+		5.7	100	2.5	4.4
Pre-Ozonation	-	.23	7.7	154	9.0	11.8
	+	.23	7.5	132	4.0	5.3
Ozonated*	-	1 ml	5.9	118	5.0	8.5
	-	.5 ml	6.4	128	6.0	9.4
	-	.25 ml	7.1	142	2.0	2.8
	-	.1 ml	6.0	120	14.0	23.3
	-	.02 ml	8.0	160	10.0	12.5
	+	1 ml	4.6	81	4.0	8.7
	+	.5 ml	6.5	114	4.0	6.1
	+	.25 ml	5.9	104	12.0	20.3
	+	.1 ml	6.2	109	13.0	21.0
	+	.02 ml	6.7	118	12.0	17.9

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*100% of starting material reacted.

Table 111

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZIDINE

EXPERIMENT 36

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.0	100	5.5	11.0
	+		5.7	100	2.5	4.4
Pre-Ozonation	-	.25 ml	6.2	124	4.0	6.5
	+	.25 ml	5.4	95	4.0	7.4
Ozonated*	-	1 ml	5.7	114	4.0	7.0
	-	.5 ml	7.1	140	3.0	4.2
	-	.25 ml	6.4	128	6.0	9.4
	-	.1 ml	5.9	118	6.0	10.2
	-	.02 ml	6.6	132	>1.0	1.5
	+	1 ml	6.5	114	5.0	7.7
	+	.5 ml	6.8	119	5.0	7.4
	+	.25 ml	5.0	88	6.0	12.0
	+	.1 ml	6.7	118	3.0	4.5
	+	.02 ml	Contam.	Contam.	Contam.	Contam.

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*100% of starting material reacted.

Table 112

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCINE

EXPERIMENT 7

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		10.0	100	4.0	4.0
	+		9.5	100	6.5	6.8
Pre-Ozonation	-	.4	7.1	71	5.0	7.0
	+	.4	9.0	95	3.0	3.3
Ozonated	-	.32	10.3	103	4.0	3.9
	-	.16	10.4	104	3.0	2.9
	-	.08	11.1	111	3.0	2.7
	-	.032	8.0	80	2.0	2.5
	-	.006	10.9	109	5.0	4.6
	+	.32	8.7	92	5.0	5.7
	+	.16	12.0	126	3.0	2.5
	+	.08	10.8	114	3.0	2.8
	+	.032	8.5	89	2.0	2.4
	+	.006	10.4	109	4.0	3.8

Table 113

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCINE

EXPERIMENT 20

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.7	100	1.0	2.2
	+		4.9	100	3.0	6.1
Pre-Ozonation	-	.48	9.4	200	2.0	2.1
	+	.48	5.1	104	1.0	2.0
Ozonated	-	.37	5.9	126	4.0	6.8
	-	.19	6.6	141	1.0	1.5
	-	.09	Contam.	Contam.	Contam.	Contam.
	-	.037	12.4	263	2.0	1.6
	-	.007	5.5	116	3.0	5.5
	+	.37	Contam.	Contam.	Contam.	Contam.
	+	.19	Contam.	Contam.	Contam.	Contam.
	+	.09	Contam.	Contam.	Contam.	Contam.
	+	.037	4.5	91	2.0	4.5
	+	.007	4.9	99	3.0	6.2

Table 114

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCINE

EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	5.5	1.7
	+		3.5	100	4.0	11.3
Pre-Ozonation	-	.44	5.8	181	2.0	3.4
	+	.44	4.3	123	5.0	11.6
Ozonated	-	.15	20.9	653	5.0	2.4
	-	.075	4.3	134	8.0	18.6
	-	.04	4.2	131	2.0	4.8
	-	.015	4.7	147	8.0	17.0
	-	.003	5.6	175	4.0	7.1
	+	.15	4.9	140	5.0	10.2
	+	.075	4.7	134	11.0	23.4
	+	.04	4.3	123	6.0	14.0
	+	.015	3.3	94	9.0	27.3
	+	.003	6.3	180	3.0	4.8

Table 115

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZENE

EXPERIMENT 4

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.8	100	6.0	10.0
	+		6.2	100	6.0	9.7
Pre-Ozonation	-	1 ml	5.2	90	3.0	5.8
	+	1 ml	5.1	82	8.0	15.7
Ozonated*	-	1 ml	4.0	70	7.0	17.5
	-	0.5 ml	4.2	72	6.0	14.3
	-	0.25 ml	5.7	98	3.0	5.3
	-	0.1 ml	5.1	88	10.0	19.6
	-	0.02	4.2	72	5.0	11.9
	+	1 ml	4.8	77	5.0	10.4
	+	0.5 ml	4.9	79	4.0	8.2
	+	0.25 ml	4.6	74	5.0	10.9
	+	0.1 ml	4.3	69	3.0	7.0
	+	0.02 ml	4.6	74	5.0	10.9

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*Percent reacted not quantitated

Table 116

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH BENZENE

EXPERIMENT 19

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.3	100	1.0	3.1
	+		5.3	100	3.0	5.7
Pre-Ozonation	-	.022				
	+	.022	8.0	152	3.0	3.7
Ozonated	-	.016	7.3	223	1.0	1.4
	-	.008	7.2	221	5.0	6.9
	-	.004	7.7	236	1.0	1.3
	-	.0016	8.7	267	<1.0	1.1
	-	.0003	6.5	200	3.0	4.6
	+	.016	Contam.	Contam.	Contam.	Contam.
	+	.008	9.2	175	1.0	1.1
	+	.004	8.4	159	2.0	2.4
	+	.0016	6.7	126	7.0	10.4
	+	.0003	8.8	168	5.0	5.7

Table 117

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CYSTEINE

EXPERIMENT 7

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml ($x\ 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($x\ 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		10.0	100	4.0	4.0
	+		9.5	100	6.5	6.8
Pre-Ozonation	-	.4	8.5	85	9.0	10.6
		.4	7.8	82	10.0	12.8
Ozonated	-	.044	10.1	100	3.0	3.0
	-	.022	9.6	96	1.3	1.4
	-	.011	8.9	89	5.0	5.6
	-	.0044	8.1	81	4.0	4.9
	-	.00088	7.8	78	5.0	6.4
	+	.044	11.0	116	7.0	6.4
	+	.022	8.4	88	5.0	6.0
	+	.011	7.8	82	6.0	7.7
	+	.0044	8.1	85	7.0	8.6
	+	.00088	8.9	94	4.0	4.5

Table 118

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CYSTEINE

EXPERIMENT 20

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.7	100	1.0	2.2
	+		4.9	100	3.0	6.1
Pre-Ozonation	-	.38	11.9	253	6.0	5.1
	+	.38	8.3	169	2.0	2.4
Ozonated	-	.31	9.2	195	2.0	2.2
	-	.15	13.5	287	4.0	3.0
	-	.075	11.8	251	1.0	0.9
	-	.031	11.0	234	6.0	5.5
	-	.006	8.1	171	1.0	1.2
	+	.31	7.2	147	3.0	4.2
	+	.15	9.5	194	3.0	3.2
	+	.075	7.9	161	1.0	1.3
	+	.031	7.2	146	2.0	2.8
	+	.006	7.9	161	5.0	6.4

Table 119

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH THYMINE

EXPERIMENT 6

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	1.9	5.9
	+		3.2	100	1.5	4.7
Pre-Ozonation	-	1.0 ml	5.6	175	7.0	12.5
	+	1.0 ml	3.5	109	5.0	14.3
Ozonation*	-	0.5 ml	5.5	172	5.0	9.1
	-	0.25 ml	4.9	153	3.0	6.1
	-	0.10 ml	3.2	100	2.0	6.3
	-	0.02 ml	4.0	125	4.0	10.0
	+	1.0 ml	3.6	113	10.0	27.8
	+	0.5 ml	4.6	144	6.0	13.0
	+	0.25 ml	5.2	163	3.0	5.8
	+	0.10 ml	6.0	188	7.0	11.7
	+	0.02 ml	4.5	141	7.0	15.6

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*100% of starting material reacted.

Table 120

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH THYMINE

EXPERIMENT 19

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.3	100	1.0	3.1
	+		5.3	100	3.0	5.7
Pre-Ozonation	-	.092	7.8	239	1.0	1.3
	+	.092	7.3	139	3.0	4.1
Ozonation	-	.006	7.2	219	10.0	13.9
	-	.003	6.5	199	3.0	4.6
	-	.0015	8.0	243	4.0	5.0
	-	.0006	7.0	213	1.0	1.4
	-	.00012	5.4	166	7.0	1.3
	+	.006	8.2	155	5.0	6.1
	+	.003	7.2	136	4.0	5.6
	+	.0015	6.6	124	8.0	12.1
	+	.0006	7.2	136	1.0	1.4
	+	.00012	8.2	155	4.0	4.9

Table 121

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CAFFEINE

EXPERIMENT 6

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	1.9	5.9
	+		3.2	100	1.5	4.7
Pre-Ozonation	-	.4	3.2	100	4.0	12.5
	+	.4	4.6	143	1.0	2.2
Ozonated	-	.25	4.0	125	4.0	10.0
	-	.13	2.6	81	10.0	38.5
	-	.06	2.9	91	3.0	10.3
	-	.025	2.7	84	5.0	18.5
	-	.005	2.7	84	3.0	11.1
	+	.25	2.8	88	7.0	25.0
	+	.13	2.2	88	7.0	31.8
	+	.06	3.9	122	3.3	8.5
	+	.025	1.7	53	2.0	11.8
	+	.005	3.7	116	1.0	2.7

Table 122

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CAFFEINE

EXPERIMENT 8

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.0	100	3.5	5.0
	+		5.8	100	5.0	8.6
Pre-Ozonation	-	.4	7.7	91	9.0	11.7
	+	.4	3.5	60	10.0	28.6
Ozonated	-	.1	6.3	90	4.0	6.3
	-	.05	7.7	110	9.0	11.7
	-	.025	4.7	67	2.0	4.3
	-	.01	5.9	84	1.0	1.7
	-	.002	7.2	103	5.0	6.9
	+	.1	6.5	112	<1.0	1.5
	+	.05	6.1	105	3.8	6.2
	+	.025	6.1	105	3.0	4.9
	+	.01	5.8	100	4.0	6.9
	+	.002	5.7	98	2.0	3.5

Table 123

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH CAFFEINE

EXPERIMENT 18

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.6	100	5.5	9.9
	+		6.2	100	6.0	9.5
Pre-Ozonation	-	.39	5.1	92	4.0	7.8
	+	.39	7.7	124	2.0	2.6
Ozonated	-	.15	4.8	87	12.0	25.0
	-	.08	6.9	124	6.0	8.7
	-	.04	5.4	97	3.0	5.6
	-	.015	6.2	112	11.0	17.7
	-	.003	5.4	97	1.0	1.9
	+	.15	5.0	80	5.0	10.0
	+	.08	8.2	132	3.0	3.7
	+	.04	4.9	79	5.0	10.2
	+	.015	6.7	109	5.0	7.4
	+	.003	6.4	103	5.0	7.9

Table 124

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIETHYLAMINE

EXPERIMENT 5

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.3	100	3.5	5.6
	+		5.4	100	3.5	6.5
Pre-Ozonation	-	.4	toxic	toxic	toxic	toxic
	+	.4	toxic	toxic	toxic	toxic
Ozonated	-	.18	toxic	toxic	toxic	toxic
	-	.09	0.7	11	1.7	24.3
	-	.045	5.5	102	8.0	14.5
	-	.018	5.3	98	6.0	11.3
	-	.0036	5.9	94	1.0	1.7
	+	.18	toxic	toxic	toxic	toxic
	+	.09	4.6	85	4.0	6.7
	+	.045	5.8	107	3.0	5.2
	+	.018	5.5	102	5.0	9.1
	+	.0036	3.8	70	1.3	3.4

Table 125

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIETHYLAMINE

EXPERIMENT 18

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.6	100	5.5	9.9
	+		6.2	100	6.0	9.5
Pre-Ozonation	-	.32	toxic	toxic	toxic	toxic
	+	.32	toxic	toxic	toxic	toxic
Ozonated	-	.20	toxic	toxic	toxic	toxic
	-	.10	5.0	90	3.0	6.0
	-	.05	5.9	105	6.0	10.2
	-	.02	4.9	88	5.0	10.2
	-	.004	5.3	94	7.0	13.2
	+	.20	toxic	toxic	toxic	toxic
	+	.10	4.4	71	4.0	9.1
	+	.05	3.4	55	3.0	8.8
	+	.02	5.0	81	6.0	12.0
	+	.04	5.7	91	5.0	8.9

Table 126

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIETHYLAMINE

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.2
	+		8.7	100	5.0	5.0
Pre-Ozonation	-	.28	toxic	toxic	toxic	toxic
	-	.14	6.4	88	2.0	3.1
	-	.07	5.3	73	6.0	11.3
	-	.03	6.3	86	3.3	5.2
	+	.28	toxic	toxic	toxic	toxic
	+	.14	5.6	64	8.0	14.3
	+	.07	6.8	78	6.0	8.8
	+	.03	7.7	89	3.8	4.9

Table 127

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH DIETHYLAMINE

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.3
	+		8.7	100	4.5	5.0
Ozonated	-	.18	toxic	toxic	toxic	toxic
	-	.09	4.0	55	4.0	10.0
	-	.05	4.0	55	5.0	13.0
	-	.02	6.4	88	3.0	4.7
	+	.18	4.0	46	5.0	13.0
	+	.09	5.3	61	6.0	11.0
	+	.05	5.8	67	4.0	6.9
	+	.02	6.3	72	6.0	9.5

Table 128

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH PHENOL

EXPERIMENT 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.4	100	5.0	9.3
	+		6.0	100	3.0	5.0
Pre-Ozonated	-	.4	6.7	124	6.0	9.0
	+	.4	6.5	108	13.0	20.0
Ozonated	-	.38	5.2	96	4.0	7.7
	-	.19	6.5	120	5.0	7.7
	-	.095	15.0	270	7.0	4.8
	-	.038	7.0	130	10.0	14.3
	-	.0076	7.1	131	2.0	2.8
	+	.38	11.0	190	8.0	7.0
	+	.19	7.2	120	6.0	8.3
	+	.095	5.0	83	4.0	8.0
	+	.038	5.6	93	3.0	5.4
	+	.0076	7.3	122	6.0	8.2

Table 129

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH PHENOL

EXPERIMENT 30

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.2	100	5.5	1.7
	+		3.5	100	4.0	11.3
Pre-Ozonated	-	.56	11.1	347	2.0	1.8
	+	.56	3.2	91	3.0	9.3
Ozonated	-	.32	Contam.	Contam.	Contam.	Contam.
	-	.16	5.9	184	150	252.9
	-	.08	12.2	381	7	5.7
	-	.032	4.1	117	7	17.2
	-	.006	Contam.	Contam.	Contam.	Contam.
	+	.32	Contam.	Contam.	Contam.	Contam.
	+	.16	7.7	220	14	18.1
	+	.08	3.8	109	1	2.7
	+	.032	4.8	137	4	8.4
	+	.006	5.3	151	5	9.5

Table 130

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH PHENOL

EXPERIMENT 31

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.4	100	3.5	8.0
	+		4.6	100	3.5	7.6
Pre-Ozonation	-	.40	5.5	125	21.0	38.2
	+	.40	4.5	98	17.0	37.8
Ozonated	-	.21	toxic	toxic	toxic	toxic
	-	.10	3.5	80	13.0	37.1
	-	.05	4.4	100	9.0	20.1
	-	.021	4.8	109	8.0	16.7
	-	.004	3.9	89	9.0	23.1
	+	.21	toxic	toxic	toxic	toxic
	+	.10	4.9	107	10.0	20.4
	+	.05	4.3	93	1.0	2.3
	+	.021	5.0	109	6.0	12.0
	+	.004	4.3	93	6.0	14.0

Table 131

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH PHENOL

EXPERIMENT 32

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.5	100	2.5	4.5
	+		6.9	100	2.0	2.9
Pre-Ozonation	-	.4	5.7	104	9.0	15.8
	+	.4	8.2	119	8.0	9.8
Ozonated	-	.38	6.5	18	1.0	1.5
	-	.19	5.8	105	12.0	20.7
	-	.095	7.6	138	8.0	10.5
	-	.038	Plates missing			
	-	.0076	5.9	107	5.0	8.5
	+	.38	7.3	106	1.0	1.4
	+	.19	5.2	75	6.0	11.5
	+	.095	7.2	104	5.0	6.9
	+	.038	Plates missing			
	+	.0076	5.1	74	2.0	3.9

Table 132

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH HYDROQUINONE

EXPERIMENT 3

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.4	100	5.0	9.3
	+		6.0	100	3.0	5.0
Pre-Ozonation	-	.4	toxic	toxic	toxic	toxic
	+	.4	toxic	toxic	toxic	toxic
Ozonated	-	.37	toxic	toxic	toxic	toxic
	-	.19	toxic	toxic	toxic	toxic
	-	.09	toxic	toxic	toxic	toxic
	-	.037	3.8	70	3.0	7.9
	-	.007	4.5	83	4.0	8.9
	+	.37	.02	.003		
	+	.19	4.7	87	3.0	6.4
	+	.09	4.3	72	4.0	9.3
	+	.037	5.5	92	1.0	1.8
	+	.007	4.3	72	3.8	8.8

Table 133

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 WITH HYDROQUINONE

EXPERIMENT 17

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.7	100	4.0	9.5
	+		6.4	100	2.5	5.1
Pre-Ozonation	-	.40	toxic	toxic	toxic	toxic
	+	.40	toxic	toxic	toxic	toxic
Ozonated	-	.30	2.1	36	105.0	500.0
	-	.15	3.9	69	4.0	10.3
	-	.04	5.0	87	8.0	16.0
	-	.03	5.7	99	3.0	5.3
	-	.006	6.6	115	2.0	3.0
	+	.30	3.1	47	34.0	109.7
	+	.15	3.9	60	3.0	7.7
	+	.04	4.4	68	3.0	6.9
	+	.03	6.3	98	1.0	1.6
	+	.006	4.8	74	1.0	2.1

Table 134

IN VITRO ASSAYS SACCHAROMYCES CEREVISIAE D3 WITH HYDROQUINONE

EXPERIMENT 29

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.2
	+		8.7	100	5.0	5.0
Pre-Ozonation	-	.59	toxic	toxic	toxic	toxic
	-	.29	toxic	toxic	toxic	toxic
	-	.15	1.9	26	2.0	11.0
	-	.06	5.8	79	3.0	5.2
	-	.01	5.5	63	5.0	9.1
	+	.59	toxic	toxic	toxic	toxic
	+	.29	3.7	17	2.0	5.4
	+	.15	5.4	62	3.0	5.6
	+	.06	5.9	68	4.0	6.8
	+	.01	4.7	54	4.0	8.5
Ozonated	-	.37	4.0	55	8.0	20.0
	-	.19	6.0	82	5.0	8.3
	-	.09	5.0	68	7.0	14.0
	-	.037	4.3	59	3.0	7.0
	-	.007	5.8	79	3.0	5.2
	+	.37	5.2	60	10.0	19.0
	+	.19	4.8	55	8.0	17.0
	+	.09	5.3	61	3.0	5.7
	+	.037	4.8	55	4.0	8.3
	+	.007	3.5	40	1.0	2.9

Table 135

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCEROL

EXPERIMENT 2

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		7.3	100	6.0	8.2
	+		9.9	100	4.5	4.5
Pre-Ozonation	-	.48	7.4	101	5.0	6.8
	+	.48	9.7	98	6.0	6.2
Ozonated	-	.48	9.1	125	3.0	3.3
	-	.24	14.0	192	6.0	4.4
	-	.12	7.9	108	3.0	3.8
	-	.048	10.0	137	7.0	7.0
	-	.0096	6.4	88	3.8	5.9
	+	.48	8.4	85	4.0	4.8
	+	.24	6.9	70	3.0	4.3
	+	.12	8.3	84	7.0	8.4
	+	.048	8.4	85	2.5	3.0
	+	.0096	8.8	89	6.0	6.8

Table 136

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCEROL

EXPERIMENT 16

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10-7)	Survivors (%)	Mitotic Recombinants per ml (x 10-3)	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		4.1	100	<1.0	2.5
	+		3.7	100	3.5	10.0
Pre-Ozonation	-	1 ml	4.5	109	3.0	6.7
	+	1 ml	4.1	112	<1.0	2.4
Ozonation*	-	1.0 ml	4.3	107	64.0	148.9
	-	0.5 ml	3.6	88	20.0	55.6
	-	0.25 ml	3.9	96	2.0	5.1
	-	0.10 ml	3.1	75	1.0	3.3
	-	0.02 ml	4.6	113	1.0	2.2
	+	1 ml	5.6	151	35.0	62.5
	+	0.5 ml	4.5	122	16.0	35.6
	+	0.25 ml	4.5	123	<1.0	2.2
	+	0.10 ml	4.1	111	2.0	4.9
	+	0.02 ml	4.2	114	1.0	2.4

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*100% of starting material reacted.

Table 137

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3
WITH GLYCEROL

EXPERIMENT 28

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		6.6	100	10.0	14.8
	+		6.1	100	6.5	10.6
Pre-Ozonation	-	.47	7.5	114	2.0	2.7
	+	.47	7.0	115	6.0	8.6
Ozonation	-	.43	12.0	182	5.0	4.2
	-	.22	7.6	115	4.0	5.3
	-	.11	8.0	121	5.0	6.3
	-	.043	6.0	91	3.8	6.3
	-	.0087	8.0	121	3.0	3.8
	+	.43	8.8	144	2.0	2.3
	+	.22	7.2	118	3.0	4.2
	+	.11	7.6	125	7.0	9.2
	+	.043	7.1	116	3.0	4.2
	+	.0087	8.2	134	6.0	7.4

Table 138

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3

ALANYLGLYCYLGLYCINE

EXPERIMENT 38

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		5.5	100	2.5	4.5
	+		6.9	100	2.0	2.9
Pre-Ozonation	-	1 ml	5.2	95	2.0	3.9
	+	1 ml	7.1	103	3.0	4.2
Ozonated	-	1 ml	6.8	124	10.0	14.7
	-	.5 ml	6.4	116	3.0	4.7
	-	.25 ml	7.9	144	6.0	7.6
	-	.1 ml	7.1	129	2.0	2.8
	-	.02 ml	5.7	104	2.0	3.5
	+	1 ml	7.8	113	4.0	5.1
	+	.5 ml	6.6	96	3.0	4.6
	+	.25 ml	7.4	107	6.0	8.1
	+	.1 ml	7.2	104	1.0	1.4
	+	.02 ml	6.0	87	3.0	5.0

Table 139

IN VITRO ASSAYS
SACCHAROMYCES CEREVISIAE D3

ALANYLGLYCYLGLYCINE

EXPERIMENT 38

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Surviving Cells per ml (x 10^{-7})	Survivors (%)	Mitotic Recombinants per ml (x 10^{-3})	Mitotic Recombinants per 10^5 Survivors
Negative Control	-		3.9	100	4.5	11.9
	+		5.6	100	5.5	10.3
Pre-ozonation	-	1 ml	6.6	169	5.0	7.5
	+	1 ml	5.4	96	8.3	15.5
Ozonated	-	1 ml	5.4	138	10.0	18.5
	-	.5 ml	4.9	126	7.0	14.3
	-	.25 ml	5.3	136	9.0	16.9
	-	.1 ml	6.0	154	5.0	8.4
	-	.02 ml	6.1	156	6.0	9.9
	+	1 ml	5.0	89	11.7	23.5
	+	.5 ml	6.2	111	10.0	16.1
	+	.25 ml	5.6	100	6.0	10.8
	+	.1 ml	6.0	107	7.0	11.6
	+	.02 ml	6.5	116	6.0	9.3

Table 140

SUMMARY OF MOLECULAR WEIGHTS AND MICROMOLES PER PLATE OF COMPOUNDS TESTED IN SALMONELLA

Compound	Molecular Weight	Highest Nontoxic Amount Tested in Salmonella	µmoles per plate
2,4-Dinitrotoluene	182	9 µg	0.05
Benzoic acid	122	2500 µg	20.5
Ethanol	46	1950 µg	42
Benzo(a)pyrene	252	unknown	
HMX	296	0.08	2.7×10^{-4}
RDX	222	7.9	0.04
Oleic acid	354	unknown	
Humic acid	--	--	--
TNT condensate water	--	--	--
1,1-Diphenylhydrazine	220.5	1131	5.1
Nitrilotriacetic acid Na_3	273	2613	9.6
Aldrin	365	unknown	
DDT	354.5	unknown	
Aroclor 1254	--	--	--
Acetic acid	53	3150	59
Glucose	180	unknown	
Urea	60	2500	42
Cholesterol	387	unknown	
Benzidine•2 HCl	257	2500	4.9
Glycine	75	2500	33
Benzene	78	137	1.8
Cysteine	121	2500	21
Thymine	126	575	4.6
Caffeine	194	2425	12.5
Diethylamine	73	2000	27
Phenol	94	3323	35
Hydroquinone	110	3663	33
Alanylglycylglycine	201	116	0.58
Glycerol	92		33